March 2003

Axoclamp-2B
MICROELECTRODE CLAMP
THEORY AND OPERATION

Part Number 2500-0115 Rev F, Printed in U.S.A.

QUESTIONS? See Axon's Knowledge Base: **http://www.axon.com/mr_Technical_Support.cfm**

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 Axoclamp-2B 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 Axoclamp-2B 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 Axoclamp-2B
The Axoclamp-2B is a solidly built instrument designed to survive shipping around the world. However, in order to avoid damage during shipping, the Axoclamp-2B must be properly packaged.

In general, the best way to package the Axoclamp-2B is in the original factory carton. If this is no longer available, we recommend that you carefully wrap the Axoclamp-2B in at least three inches (75 mm) of foam or "bubble-pack" sheeting. The wrapped Axoclamp-2B 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 Axoclamp-2B. If the carton is dropped by the shipper, there is a good chance that the Axoclamp-2B will shift within the loose pellet packaging and be damaged.

If you need to ship your Axoclamp-2B 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.

RENSEIGNMENTS IMPORTANTS

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 Axoclamp-2B 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 Axoclamp-2B 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".

Précautions de sécurité
Dans le mode TEVC le appareil electronique peut vous donner un pulse de haut voltage qui est limitée pour l'alimentation seulement a le connecteur des electrodes de la tête de l'amplificateur.

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

ATTENTION
Expédition de le Axoclamp-2B
Le Axoclamp-2B 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 Axoclamp-2B est de le mettre dans son carton d'origine. Si celui-ci n'est plus disponible, il est recommandé d'envelopper soigneusement le Axoclamp-2B dans au moins trois inches (75 mm) de mousse ou de feuilles d'emballage à bulles. Le Axoclamp-2B 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 Axoclamp-2B. En cas de chute de la boîte durant son transport, le Axoclamp-2B pourrait se déplacer à l'intérieur et être endommagé.

Si vous devez expédier le Axoclamp-2B 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 Axoclamp-2B 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 Axoclamp-2B 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 Axoclamp-2B
Bei dem Axoclamp-2B handelt es sich um ein solide gebautes Instrument, das beim weltweiten Versand keinen Schaden nehmen sollte. Um jedoch Versandschäden zu verhindern, muß der Axoclamp-2B ordnungsgemäß verpackt werden.

Im allgemeinen läßt sich der Axoclamp-2B am besten im Originalkarton des Werks verpacken. Ist dieser nicht mehr vorhanden, empfehlen wir, den Axoclamp-2B vorsichtig in mindestens 75 mm starkem Schaumstoff oder Bubblepackungen einzuwickeln. Der so eingewickelte Axoclamp-2B 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 Axoclamp-2B innerhalt der losen Schaumstoffkugelverpackung bewegt wird und dadurch beschädigt werden kann.

Wenn Sie den Axoclamp-2B 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.

INFORMACION IMPORTANTE

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 Axoclamp-2B 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 Axoclamp-2B 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 electrónico de ME2 puede generar un pulso de alto voltaje limitado solamente por corriente al conector del cabezal del electródo.
Para su seguridad, no toque el punto del electródo mientras usando el modo TEVC.

ADVERTENCIA
Envío del Axoclamp-2B
El Axoclamp-2B 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 Axoclamp-2B debe empacarse adecuadamente.

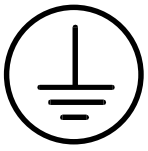
En general, la mejor manera de empacar el Axoclamp-2B es en la caja original de fábrica. Si ésta ya no se encuentra disponible, le recomendamos que envuelva cuidadosamente el Axoclamp-2B en una funda o sábana de espuma o de "empaque de burbujas" con un espesor mínimo de 3 pulgadas (75 mm). El Axoclamp-2B, 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 Axoclamp-2B. Si la caja se cae accidentalmente durante el transporte, es muy probable que el Axoclamp-2B se desplace dentro del contenedor con las bolitas de espuma sueltas y se dañe.

Si necesita enviar su Axoclamp-2B 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.

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

February 2003

ADDENDUM:
VOLTAGE-CLAMP STEADY-STATE RESTORE

IMPORTANT NOTICE: We recommend that you perform the entire Functional Checkout and Tutorials described in this Axoclamp-2B Theory and Operation Manual with the rear-panel "Voltage Clamp Steady-State Restore" switch in the OFF position. After completion of the Tutorials, then perform the "TEVC Performance Verification" described below.

Although this Theory and Operation manual refers to the MCO-1U model cell, you will be using the MCO-2U model cell for your tests. The MCO-2U in the "1M" position is identical to the MCO-1U.

The "*Steady-State Restore*" feature was designed into all Axoclamp-2B units beginning February 2003. Please see the related Application Note on our Axoclamp web page for more detailed information (http://www.axon.com/CN_Axoclamp2B.html). This modification increases the two-electrode voltage-clamp (TEVC) gain in order to account for voltage errors that occur when large cells such as oocytes pass very high current (many tens of μA), during which time the membrane resistance may drop to very low levels (a few kΩ). If your TEVC recordings do not produce these extreme conditions of high current/low membrane resistance, then you will not benefit from the *Steady-State Restore* enhancement.

If you own an Axoclamp-2B manufactured prior to February 2003 and wish to modify your unit with the *Steady-State Restore* feature, contact Axon Technical Support (tech@axon.com or 510-675-6200) for information.

TEVC Performance Verification

The following procedure describes a specific verification test of the TEVC *Steady-State Restore* function using the MCO-2U model cell. This test should be performed after you have verified the basic function of the Axoclamp-2B via the Functional Checkout procedure described in the user manual.

1. Switch rear panel *Voltage Clamp Steady State Restore* OFF
2. Connect HS-2A-x1LU to ME1 and HS-2A-x10MGU to ME2
3. Connect headstages to MCO-2U model cell (with switch in "1M" position)
4. Connect 4-lead wire assembly to:
    a. MCO-2U CELL input
    b. MCO-2U CASE input
    c. ME1 ground socket
    d. Shield
5. I Display Select=0.1 x I2

6. H1=1; H2=10; VG=1
7. Rate Adjust=fully CCW
8. Step Command=+100 mV; switched OFF; Destination=VC
9. Select Bridge Mode
10. Voltage Clamp section:
    a. Anti-alias filter fully CCW
    b. Gain: 25
    c. Phase Lag: 0.15
    d. Multiplier x 1
    e. Holding Position=5.0
11. ME1/ME2 sections:
    a. Cap Neut=fully CCW
    b. Bridge=fully CCW
    c. DC Current Cmd=fully CCW
    d. Adjust Input Offset to zero Vm meter (ME1) and V2 meter (ME2)
12. Select TEVC mode
13. Vm meter should read 000 mV
14. I (nA) meter should read 000 nA (adjust ME1 input offset if necessary)
15. Set STEP COMMAND activation switch to CONT to give a +100 mV command
16. Vm meter should read 100 mV (+/-2 mV) and I (nA) meter should read 10 nA (+/-1 nA). Remember that this is monitoring 0.1I2 so actual current is 100 nA
17. Switch MCO-2 switch to the 10k position
18. Vm meter should read 88 to 95 mV
19. I (nA) meter should read 800 to 900 nA
20. Switch rear panel *Voltage Clamp Steady State Restore* ON
21. Vm meter should read 100 mV (+/-2 mV)
22. I (nA) meter should read 870 to 970 nA

NOTE: If you find that the *Steady-State Restore* function does not behave as the verification instructions indicate, it may be that the switch was installed in the opposite polarity. Repeat the test above, but begin with the Steady-State Restore switch ON (and of course, turned OFF in Step #20).

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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.

DISCLAIMER

THIS EQUIPMENT IS NOT INTENDED TO BE USED AND SHOULD NOT BE USED IN HUMAN EXPERIMENTATION OR APPLIED TO HUMANS IN ANY WAY.

Illustrations of the front-panel and rear-panel views of the Axoclamp-2B are shown on the fold-out at the rear of the manual.

TABLE OF CONTENTS

LIST OF FIGURES x

INTRODUCTION 1

SAFETY PRECAUTIONS 3

FUNCTIONAL CHECKOUT 5
- Start-Up Procedure 5
- Testing the Clamp 6

uSING THE AXOCLAMP-2b — TUTORIALS 9
- Summary of Controls, Inputs and Outputs 9
  - Mode Group 9
  - Microelectrode 1 (ME1) Group 9
  - Microelectrode 2 (ME2) Group 10
  - Voltage-Clamp Group 10
  - Step-Command Group 10
  - Rate Group 11
  - Outputs 11
  - Inputs 12
  - Remote 12
  - Initial Instrument Settings (Default) 12
- Bridge Mode 13
  - Headstage Selection 13
  - Connections 13
  - Acquisition and Command Setup 13
  - Balance the Bridge in the 14
  - Balance the Bridge in the 16
- Continuous Single-Electrode Voltage Clamp (cSEVC) Mode 17
  - Headstage Selection 17
  - Initial Instrument Settings 17
  - Connections 17
  - Acquisition and Command Setup 17

Balance the Bridge 17
Clamp the ″Cell″ 18
Discontinuous Current Clamp Mode 20
Headstage Selection 20
Initial Instrument Setting 20
Connections 20
Acquisition and Command Setup 20
Adjust the Sample Rate and the Capacitance Neutralization 21
Discontinuous Single-Electrode Voltage Clamp Mode - dSEVC 23
Headstage Selection 23
Initial Instrument Settings 23
Connections 23
Acquisition and Command Setup 23
Adjust the Sample Rate and Capacity Neutralization 24
Clamp the ″Cell″ 24
False Clamp 25
TEVC Mode 27
Headstage Selection 27
Initial Instrument Settings 27
Connections 27
Acquisition and Command Setup 28
Balance the Bridge 28
Tune the Voltage Clamp 28

MICROELECTRODES 31
Microelectrodes for Fast Settling 31
Microelectrode Capacitance 31
Target Cell Near Surface of Solution 31
Target Cell Deep in Solution 32
Capacity Transients and Solution Levels 32
Micropipette or Patch Pipette? 33
Micropipettes 33
Electrode Glass 33
Tip Potentials — Detection 33

Tip Potentials — Prevention 34
Tip Resistance 34
Settling Time 34
Stability 34
Filling Solutions 34
Cell Impalement 35
Patch Pipettes 36
Glass Type and Coating 36
Noise 36
Seal Formation and Whole Cell Recording Configuration 37

REFERENCE GUIDE: GENERAL InFORMATION 39
Interfacing a Computer to the Axoclamp-2B 39
Grounding and Hum 40
Power-Supply Glitches 41
Model Cells 41
The CLAMP-1U Model Cell 41
The MCW-1U Model Cell 42
Installation of Model Cells 43
Ten-turn Potentiometers 44

REFERENCE GUIDE: INSTRUMENT OPERATION 45
Blanking 45
Buzz 45
Calibration Signal 46
Clear 47
Command Generators 47
Step Command Generator 47
DC Command Generators 48
External Command Inputs 48
Mixing Commands 48
Headstages 49
HS-2A and HS-2 Series 49
HS-4 Series 50
VG-2 Series 50

VG-2A-x100 Series 50
Holder 51
Features 51
Parts 51
Use 52
Insertion of Pipette 52
Cleaning 52
Filling Pipette 52
Silver Chloriding 53
Replacing the Silver Wire 53
Glass Dimensions 53
Adapters 53
Ionophoresis 54
Link-Up 54
Monitor 55
Output Impedance and Protection 55
Panel Meters 56
Power Supply Voltage Selection & Fuse Changing 56
Remote Control 56
RMP Balance 58
Triggered Clamping 58

REFERENCE GUIDE: PRINCIPLES OF OPERATION 59
Anti-Alias Filter 59
Bath Error Potentials 59
Minimize R_b 60
Clamp V_b Using a Bath Clamp or Virtual Ground 61
Independently Measure V_b and Subtract it from the ME1 Potential 61
Grounding 62
Bandwidth of V_b Measurement 62
Compensate the Series Resistance 62
Summary 63
Capacitance Neutralization and Input Capacitance 64
Primary Method of Neutralizing C_{in} 64
Secondary Method for Neutralizing C_{in} 64

Grounding and Driven Shield 65
Current Measurement 65
Current and Voltage Conventions 66
Whole-Cell Voltage and Current Clamp 66
Depolarizing / Hyperpolarizing Commands 66
Transmembrane Potential vs. Command Potential 67
Inward / Outward Current 67
Using cSEVC in a Macropatch Configuration 67
Cell-Attached Macropatch 67
Inside-Out Macropatch 68
Summary 68
Filtering with External Filters 68
Headstages 69
Compatibility of the HS-2 and HS-2A With the Axoclamp-2B 69
General Characteristics 69
The Meaning of H 69
Guidelines for Selecting a Headstage 70
Capacitance Neutralization Range 70
General Rule for Choosing an HS-2(A) Headstage 71
HS-4-x1MGU Headstage 72
Warnings 73
Headstage Connectors 73
Microelectrode Input Connector (White) 73
Shield Drive Connector (Gold) 73
Ground Output Connector (Yellow) 74
Interchangeability 74
Cleaning 74
Input Leakage Current 74
How To Trim the Input Leakage Current /to Zero 75
Warning 75
DC Removal 76
Input Resistance 76
Offset Controls 76
Output Filter 77
High-Order Low-Pass Filters for Low-Noise Recording 77

Rise Time of High-Order FIlters 77
Note on Ultimate Rise Time 78
Phase Lag 78
Use 78
Series Resistance 78
Unity-Gain Recording — Third Point 79
Virtual-Ground Current Measurement 79
10 V_m And I_m Outputs 80

REFERENCE GUIDE: THEORY OF RECORDING MODES 81
Bridge Mode 81
Continuous Single-Electrode Voltage Clamp 81
Axoclamp-2B cSEVC Mode Compared to a Patch-Clamp Amplifier 82
Pipette Capacitance Compensation 83
Series Resistance Compensation 83
Advantages of Whole-Cell Recording With a Patch Pipette 84
Macropatch Technique 85
Discontinuous Current Clamp 85
Discontinuous Single-Electrode Voltage Clamp (dSEVC) Mode 87
Minimum Sampling Rate And Maximum Gain 89
Clamp Error 90
Gain 90
Series Resistance 90
Advantages of a Patch Pipette 90
dSEVC and cSEVC Compared 91
Noise in DCC and dSEVC Modes 93
TEVC Mode 94
General Considerations 95
Configuration of the Voltage Clamp System 95
Voltage Recording and Series Resistance 95
Selection of the Membrane Voltage Recording Headstage 96
Selection of the Current Recording Method 96
Series Current Measurement (Internal) 96
Virtual Ground Current Measurement 97
Tuning the Voltage-Clamp — Fidelity and Stability 98

Fidelity 98
Step Response and Bandwidth 98
Stability 98
Inter-Electrode Coupling Capacitance and Shielding 99
Eliminating the Voltage Error Due to Coupling Between Intracellular Microelectrodes 100

TROUBLE SHOOTING 101
Voltage Clamp Problems 101
cSEVC 101
dSEVC 101
TEVC 101
All Voltage Clamp Modes 102
Space Clamp 103
Noise 104

SPECIFICATIONS 105

REFERENCES 113

STANDARD WARRANTY AND REPAIR SERVICE 115

ADVISORY REGARDING SHIPPING 117

DECLARATION OF CONFORMITY 119

CIRCUIT DIAGRAMS REQUEST 121

GLOSSARY 123

INDEX 125

LIST OF FIGURES

Page

Figure 1. Bridge Balancing Procedure 15

Figure 2. Correctly Adjusted Bridge and Capacitance Neutralization Controls Using the CLAMP-1U Model Cell 16

Figure 3. Current and Potential Recording During cSEVC in a Cell Model 19

Figure 4. How to Set the Capacitance Neutralization During DCC Mode 22

Figure 5. Correctly Set Up dSEVC in a Cell Model 25

Figure 6. Incorrectly Set Up dSEVC in a Cell Model 26

Figure 7. Tuning the TEVC with the CLAMP-1U Model Cell 29

Figure 8. Tuning the TEVC with the MCO-1U Model Cell 30

Figure 9. Two-Phase Microelectrode Decay 32

Figure 10. CLAMP-1U Model Cell 42

Figure 11. MCW-1U Model Cell 42

Figure 12. Suggested Cell Model 43

Figure 13. Summation of Commands 49

Figure 14. HS-2 and HS-4 Headstage Connection Diagram 50

Figure 15. Exploded View of the Holder 51

Figure 16. Ag/AgCl Pellet Assembly 52

Figure 17. Anti-Alias Filter and Monitor Circuit 55

Figure 18. External Switch Wiring Diagram 57

Figure 19. Bath Clamp 61

Figure 20. Simplified Schematic of cSEVC 82

Figure 21. Recordings from Brain Slices in Bridge Mode and cSEVC Mode 84

Figure 22. DCC Mode Block Diagram and Timing Diagram 86

Figure 23. dSEVC Block Diagram and Timing Waveforms 89

Figure 24. Comparison of Current and Potential During dSEVC and cSEVC Using the MCW-1U Model Cell 92

INTRODUCTION

The Axoclamp-2B is a complete microelectrode current and voltage clamp for intracellular investigations. It combines state-of-the-art single-electrode voltage clamping, two-electrode voltage clamping, and two complete bridge amplifiers into one instrument.

Voltage clamping is a powerful technique for the control of membrane potential and for the investigation of processes affecting membrane conductance. The choice of using a voltage clamp with one or two microelectrodes is dictated in large part by the preparation, as well as by the needs of the investigation. The advantages of single-electrode over two-electrode techniques are the technical ease of placing a single microelectrode in cells that are small or difficult to visualize, and that the instability due to crosstalk (coupling capacitance and coupling resistance) between two microelectrodes does not occur. The high compliance two-electrode voltage technique is needed for large cells (such as amphibian oocytes) with currents too large to clamp with a single electrode.

The design of the Axoclamp-2B reduces the disadvantages of single-electrode techniques towards their theoretical minimums, thereby allowing single-electrode voltage clamping to be performed in the many situations where conventional two-electrode voltage clamping is not feasible. This advanced design is unlikely to limit the achievable performance. Users of the Axoclamp-2B in either of the SEVC modes should be quick to question, then adjust, the microelectrode and its placement.

Continuous Single-Electrode Voltage Clamp (cSEVC) uses a low resistance electrode simultaneously to record membrane potential and inject current. The disadvantage of this technique is that a systematic error in the measured voltage is introduced by the voltage drop across the microelectrode resistance. This can be partially reduced by series resistance compensation. Since the required compensation is never perfect, the cSEVC mode is useful only when the microelectrode resistance is very small compared with the cell input resistance, a condition achieved with a patch pipette. The cSEVC technique is as low in noise as the two-electrode voltage-clamp technique.

Discontinuous Single-Electrode Voltage Clamp (dSEVC) is based on the technique of sampling the membrane potential while zero current flows and then retaining this sampled value while current is injected into the cell. This procedure is rapidly repeated to produce a smooth response. A particular advantage of a dSEVC is that the voltage drop due to current flow through the series component of electrode membrane resistance (R_s) is not clamped. However, compared to the cSEVC mode, the dSEVC mode has higher noise.

Two-Electrode Voltage Clamp (TEVC) uses two microelectrodes, one dedicated for the continuous recording of membrane potential, and a second electrode for the injection of current. In TEVC mode the compliance of the Axoclamp-2B is ±130 V, offering the ability to clamp large currents that are impossible to control with single-electrode techniques. Additional advantages of TEVC mode are that the noise can be low, the response speed is fast, and the maximum achievable gain is high.

The current clamp technique is a method for observing membrane voltage responses without clamping the membrane potential. The membrane potential of the cell is not clamped. Instead, the regulated injection of current provides a means to evoke membrane voltage responses by depolarizing or hyperpolarizing current steps and observe either postsynaptic potentials and action potentials.

In Bridge mode a single electrode is used to simultaneously inject current and measure the resulting change in membrane potential. In contrast, Discontinuous Current Clamp (DCC) mode uses a single

microelectrode cyclically to pass current and to measure the voltage of the cell. The voltage recorded at the tip of the microelectrode is memorized by a sample-and-hold circuit during intervals between the current-passing periods. Thus the membrane potential can be recorded independently of the voltage drop across the microelectrode resistance. The advantage of DCC mode over the Bridge mode (an alternative method for intracellular recording) is that DCC mode is tolerant of small changes in microelectrode resistance. A disadvantage is that DCC mode is noisier than the Bridge mode.

The Axoclamp-2B is a sophisticated instrument. Experienced and inexperienced researchers alike are advised to read this manual thoroughly and to familiarize themselves with the instrument using model electrodes (*i.e.,* resistors) and cells (*e.g.,* parallel RC) before attempting experiments with real microelectrodes and cells. Model cells are provided with the basic equipment.

We will be pleased to answer any questions regarding the theory and use of the Axoclamp-2B. Any comments and suggestions on the use and design of the Axoclamp-2B will be much appreciated. We welcome reprints of papers describing work performed with the Axoclamp-2B. Keeping abreast of research performed helps us to design our instruments to be of maximum usefulness to you who use them.

Axon Instruments, Inc.

NOTES

The Axoclamp-2B 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 Axoclamp-2B, 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.

Throughout this manual, "microelectrode" is used synonymously with either a "patch pipette" or "micropipette." "Micropipette" refers to a sharp intracellular glass electrode.

References to the front panel 10 V_m,I_m OUTPUT BANDWIDTH and I_B are equivalent to V_m,I_m OUTPUT BANDWIDTH and I_{VG}.

SAFETY PRECAUTIONS

Applicability

The Axoclamp-2B can accept several different types of headstages for microelectrode 1 (ME1) and microelectrode 2 (ME2). 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.

Safety Recommendations

Please follow these advisories:

1) Never touch the headstage case when the Axoclamp-2B is switched on.

2) Turn down the capacitance neutralization controls to their minimum settings (fully counter-clockwise) before approaching the headstage.

3) Switch back to BRIDGE mode before approaching the ME2 **headstage case**.

The maximum current that can be delivered by an HS-4 headstage in the ME2 position is ±10 mA. To avoid possible contact with the current via the electrode connector in an HS-4 series headstage used in the ME2 position:

1) Switch back to BRIDGE mode before touching the **electrode holder**.

ME1 Background

The output of the ME1 capacitance neutralization circuit is limited to about ±35 V. Transient voltages approaching these ±35 V limits can be expected to occur during normal operation. For example, during discontinuous single-electrode voltage clamp (dSEVC), electrode current transients can cause the voltage at the headstage input to reach ±13 V. If the capacitance neutralization control is near its maximum setting, these transient voltages can result in ±35 V transients at the output of the capacitance neutralization circuit and therefore ±35 V transients on the headstage case. Similarly, if the capacitance neutralization circuit oscillates (as might happen after the microelectrode is withdrawn from the cell or bath), the oscillating voltage on the headstage case could reach ±35 V.

SAFETY PRECAUTIONS

ME2 Background

The output of the ME2 capacitance neutralization circuit is limited to about ±160 V. Transient voltages approaching these ±160 V limits can be expected to occur during normal operation. For example, during two electrode voltage clamp (TEVC) the membrane capacitance transients can cause the voltage at the headstage output to transiently reach over 100 V. If the capacitance neutralization control is well advanced, these transient voltages can result in ±160 V transients at the output of the capacitance neutralization circuit and therefore ±160 V transients on the headstage case. Similarly, if the capacitance neutralization circuit oscillates (as might happen after the microelectrode is withdrawn from the cell or bath), the oscillating voltage on the headstage case could reach ±160 V. Finally, if the Axoclamp-2B is in TEVC mode and 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.

A further consideration for the ME2 headstage is that during TEVC 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. The other headstages listed above have larger resistor values, thus, with one exception, the current is limited to an even smaller value in these headstages. The exception is that the current that can be delivered via the electrode connector in an HS-4 series headstage (*e.g.,* HS-4-x1MGU) during TEVC mode can be up to ±10 mA. Note that during any other operating mode, the current that can be delivered via the electrode connector in an HS-4 series headstage is limited by a 10 MΩ resistor.

Safety Circuits

The Axoclamp-2B implements the following safety circuits.

1) The headstage case is connected to the capacitance neutralization circuit via an .0047 µF capacitor inside the headstage. Thus under conditions where the capacitance neutralization circuit is steady at plus or minus 160 V (for ME2) or 35 V (for ME1), only a single transient current can be discharged from the headstage case.

2) 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 ME2 position, the current is limited to ±10 mA. For all other headstages listed above, and for the HS-4 series headstage in the ME1 position, the current is limited to less than ±1 mA.

FUNCTIONAL CHECKOUT

Start-Up Procedure

For the initial checkout, the Axoclamp-2B should be situated on a bench top away from other equipment. Do not install it in a rack until the checkout is complete. An oscilloscope, a signal source for generating a square-wave (TTL pulse), and two BNC cables are the only other pieces of equipment required for these tests. A large sheet of aluminum foil can be used to shield the headstages and the CLAMP-1U model cell. Make sure that the power is OFF.

This functional checkout uses the HS-2A-x0.1LU and HS-2A-x1LU headstages shipped standard with the Axoclamp-2B. For other suitable headstages see *Headstages* in **REFERENCE GUIDE: PRINCIPLES OF OPERATION**.

Initial Instrument Settings

Before starting, set the panel controls to the "default" settings indicated below. Note, **Minimum** values are fully counterclockwise.

Step Command Group

Step Command Thumbwheel - **Zero**
Ext/Cont/Off Switch - **Off**
Destination Switch - **ME1**

Microelectrode (ME1) and (ME2) Groups

Capacitance Neutralization - **Minimum**
Bridge - **Zero**
ME1: Input Offset - **None** (≈5)
ME2: Output Offset - **None** (≈5)
DC current command - **Switch OFF**

I Display Select

I_m

Headstage Gain Selectors

H1 - 0.1
H2 - 1.0

Rate Adjust

Minimum

Voltage Clamp Group

Anti-Alias - **Minimum**
Gain - **Minimum**
Phase Lag - **0.01**
Phase Multiplier - **OFF**
Holding Position - **Any desired level**

10 V_m, I_m Output Bandwidth

30 kHz

Testing the Clamp

Testing Bridge Mode

Plug the HS-2A-x0.1LU headstage into the connector on the rear panel of the Axoclamp-2B labeled ME1 PROBE and the HS-2A-x1LU headstage into the connector labeled ME2 PROBE. Connect the two headstages to the CLAMP-1U model cell supplied with the Axoclamp-2B as indicated on the model cell and set the model cell switch to BATH. Connect the gold-plated grounding jack on the model cell to the yellow jack at the rear of the ME1 headstage using the cable provided. Insert one end of a two-lead connector into the yellow socket at the rear of the ME2 headstage and the other end of the lead to the aluminum foil. Turn on the Axoclamp-2B.

Ensure that the STEP COMMAND toggle switch is in the OFF position. The digital panel meters V_m and V_2 should read 000 within 1 mV. If not, use the INPUT OFFSET dial and the OUTPUT OFFSET dial to zero the V_m and V_2 digital panel meter, respectively. Set the H1 switch to indicate the gain of the headstage on ME1 (x0.1) and set the H2 switch to indicate the gain of the headstage used on ME2 (x1). Using the I DISPLAY SELECT switch, select I_m and confirm that the I(nA) digital panel meter reads 0.00 nA within 0.01 nA. Turn all controls not mentioned above to their full counterclockwise position. Set the model cell switch to the **CELL** position.

ME1:

Check that the 10 V_m,I_m OUTPUT BANDWIDTH switch is set to 30 kHz. Monitor the I_m output with the oscilloscope. Set the oscilloscope time base to 10 ms/div, the gain to 0.5 V/div and trigger to line. Set the STEP COMMAND thumbwheel to 050.0, the STEP COMMAND DESTINATION switch to ME1, and the STEP COMMAND toggle switch to CONT. Alternate the thumbwheel switch polarity between "+" and "-" positions. You should observe a positive or negative 0.5 V DC trace on the oscilloscope. The current digital panel meter should read 5.00 ± 0.01 nA with the polarity corresponding to that set on the thumbwheel switch. Check that the green LED below the ME1 DC CURRENT COMMAND dial illuminates. Set the oscilloscope gain to 2 V/div. Set the STEP COMMAND toggle to OFF. Toggle the ME1 CLEAR switch first to (+) and then to (-). A trace of approximately 5 V DC with polarity corresponding to that of the CLEAR switch should appear on the oscilloscope. Connect the output of the square wave generator to the STEP ACTIVATE input on the rear panel of the Axoclamp-2B. Set the generator to 100 Hz and the STEP COMMAND toggle switch to EXT. Trigger the oscilloscope with the square wave generator. Set the oscilloscope gain to 0.5 V/div. A 0.5 V, 100 Hz square wave with a polarity matching that set on the thumbwheel switch should appear on the oscilloscope.

Use the oscilloscope to observe the 10 V_m output. Set the oscilloscope gain to 1 V/div, the time base to 0.2 ms/div and the trigger to internal. Set the generator to 500 Hz. Adjust the ME1 BRIDGE dial until the voltage steps at the leading and trailing edges of the voltage response are eliminated, leaving residual capacity transients. The BRIDGE dial should read 50 MΩ when properly adjusted. Adjust the ME1 CAPACITANCE NEUTRALIZATION knob so that the residual capacity transients narrow and settle to baseline without overshooting or ringing (approximately 2 to 3 turns). The settling time should be less than 80 µs. You should observe high frequency oscillations on the oscilloscope when you press the ME1 BUZZ push-button. Return the CAPACITANCE NEUTRALIZATION and BRIDGE controls to their minimums and set the STEP COMMAND toggle switch to OFF.

ME2:

Use the oscilloscope to monitor the I_2 output on the rear panel. Set the oscilloscope time base to 10 ms/div , the gain to 0.5 V/div and the trigger to line. Select 0.1 x I_2 on the I DISPLAY SELECT switch and confirm that the I(nA) digital panel meter reads 00.0 nA within 0.1 nA. Set the STEP COMMAND thumbwheel to 050.0, the STEP COMMAND DESTINATION switch to ME2, and the STEP COMMAND toggle switch to CONT. Alternate the thumbwheel switch polarity between (+) and (-) positions. You should observe a positive or negative 0.5 V DC trace on the oscilloscope. The current digital panel meter should read 5.0 ± 0.1 nA with the polarity corresponding to that set on the thumbwheel switch. Check that the green LED below the ME2 DC CURRENT COMMAND dial illuminates. Set the STEP COMMAND toggle to OFF. Toggle the ME2 CLEAR switch first to (+) and then to (-). A trace of approximately 1 V DC with polarity corresponding to that of the CLEAR switch should appear on the oscilloscope. Connect the output of the square wave generator to the STEP ACTIVATE input on the rear panel of the Axoclamp-2B. Set the generator to 100 Hz and the STEP COMMAND toggle switch to EXT. Trigger the oscilloscope with the square wave generator. A 0.5 V, 100 Hz, square wave with a polarity matching that set on the thumbwheel switch should appear on the oscilloscope.

Use the oscilloscope to observe the V_2 output. Set the oscilloscope gain to 1 V/div, the time base to 0.2 ms/div and the trigger to internal. Set the generator to 500 Hz. Adjust the ME2 BRIDGE dial until the voltage steps at the leading and trailing edges of the voltage response are eliminated, leaving residual capacity transients. The BRIDGE dial should read 50 MΩ when properly adjusted. Adjust the ME2 CAPACITANCE NEUTRALIZATION knob so that the residual capacity transients narrow and settle to baseline without overshooting or ringing. The settling time should be less than 80 µs. You should observe high frequency oscillations on the oscilloscope when you press the ME2 BUZZ push-button. Return the CAPACITANCE NEUTRALIZATION and BRIDGE controls to their minimums and set the STEP COMMAND toggle switch to OFF.

Testing DCC Mode

While still in BRIDGE mode, set the STEP COMMAND DESTINATION switch to ME1 and the STEP COMMAND toggle switch to EXT. Monitor the 10 V_m output with the oscilloscope. Set the oscilloscope gain to 1 V/div, the time base to 5 ms/div and the trigger to internal. Set the generator to 50 Hz. With the STEP COMMAND thumbwheel set to 050.0, adjust the ME1 BRIDGE dial until the voltage steps at the leading and trailing edges of the voltage response are eliminated, leaving narrow transients. Adjust the ME1 CAPACITANCE NEUTRALIZATION knob so that the residual capacity transients narrow and settle to baseline without overshooting.

Ensure that the RATE ADJUST knob is turned to its minimum setting (fully counterclockwise). Switch to DCC mode by pressing the yellow DCC push button. Slowly increase the RATE ADJUST knob until the same voltage waveform as seen in BRIDGE mode appears without the fast transients. Continue to increase the sampling rate until the voltage waveform begins to separate at the voltage transitions and then reduce the sampling frequency slightly. The resultant sampling frequency should be between 7 and 8 kHz and the voltage waveform should be a smooth representation of the same response in BRIDGE mode but without the fast transients (this can be confirmed by alternately switching between BRIDGE and DCC). Return to DCC mode.

Use the oscilloscope to monitor the I_m OUTPUT. A square wave with a peak-to peak amplitude of 0.5 V, a frequency of 50 Hz, and polarity matching that of the STEP COMMAND thumbwheel switch should appear. Before proceeding to the next test, set the STEP COMMAND toggle switch to OFF.

Testing dSEVC Mode

The ME1 Capacitance Neutralization and Rate Adjust settings should be the same as those used in the above test of the DCC mode. Monitor the 10 V_m output with the oscilloscope. Ensure that the voltage clamp Gain and Anti-Alias Filter are set to their minimum settings (fully counterclockwise). Adjust the Holding Position dial so that both the RMP Balance LEDs are equally dim. Set the 10 V_m, I_m Output Bandwidth to 1 kHz.

Switch to dSEVC mode by setting the MODE toggle switch to DISCONT. SEVC and pressing the red SEVC push-button. Set the STEP COMMAND DESTINATION switch to VC, the STEP COMMAND toggle switch to EXT. and increase the voltage clamp GAIN to 0.8 nA/mV. The signal from the 10 V_m output signal should be a square wave with a peak-to-peak amplitude of 500 mV and a rise time less than 1 ms. Adjust the ME1 CAPACITANCE NEUTRALIZATION knob to eliminate the small voltage spikes that occur at the rising and falling edges of the voltage response. Set the voltage-clamp GAIN to its minimum (fully counterclockwise). Return the ME1 BRIDGE and the ME1 CAPACITANCE NEUTRALIZATION controls to their minimum settings (fully counterclockwise) and the 10 V_m, I_m Output Bandwidth switch to 30 kHz.. Return to BRIDGE mode.

Testing cSEVC Mode

In BRIDGE mode set the STEP COMMAND DESTINATION switch to VC, the STEP COMMAND toggle switch to OFF and the STEP COMMAND thumbwheel switch to +050.0. Ensure that the voltage clamp GAIN and ANTI-ALIAS FILTER are turned to their minimum settings (fully counterclockwise). Set the I DISPLAY SELECT switch to I_m. Adjust the HOLDING POTENTIAL dial so that both RMP BALANCE LEDs are equally dim.

Switch to cSEVC mode by setting the MODE toggle switch to CONT. SEVC and pressing the red SEVC push-button. Set the STEP COMMAND toggle switch to CONT. and check that the V_m(mV) digital panel meter reads 50 mV ±1 mV, the I (nA) Digital Panel Meter reads 0.50 nA ± 0.01 nA, and the red LED near the ME1 BRIDGE dial is on. Set the STEP COMMAND toggle switch to OFF. Return to BRIDGE mode.

Testing TEVC Mode

In BRIDGE mode set the STEP COMMAND thumbwheel to +050.0. Turn both ME1 and ME2 CAPACITANCE NEUTRALIZATION to their minimum settings (fully counterclockwise). Select 0.1 x I_2 with the I DISPLAY SELECT switch. Turn the ME2 BRIDGE dial, the voltage-clamp GAIN and ANTI-ALIAS FILTER to their minimum settings (fully counterclockwise). With the STEP COMMAND off, adjust the HOLDING POSITION dial so that both the RMP BALANCE LEDs are equally dim. Set the STEP COMMAND DESTINATION switch to VC and the STEP COMMAND toggle switch to CONT. Switch to TEVC mode by pressing the blue TEVC push button. Increase the voltage clamp GAIN to 150 V/V. The V_m(mV) and I(nA) digital panel meters should display 50 mV ±1 mV and 0.1 nA, respectively.

Use the oscilloscope to monitor the 0.1 x I_2 output. Set the oscilloscope to 0.5 V/div, the time base to 0.2 ms/div and the trigger to internal. Set the generator to 500 Hz. Set the STEP COMMAND toggle switch to EXT. Current transients approximately 2 V peak-to-peak will appear. Eliminate the over-shoot and ringing by interactively adjusting the ME1 CAPACITANCE NEUTRALIZATION and ME2 CAPACITANCE NEUTRALIZATION. It should be possible to achieve a settling time less than 60 µs.

Use the oscilloscope to monitor the 10 V_m output. Adjust the oscilloscope gain to 0.1 V/div. A square wave with an amplitude of 500 mV and a rise time less than 200 µs should appear.

USING THE AXOCLAMP-2B — TUTORIALS

It is recommended that you set up and test the electronics using the model cell supplied, or one of your own design that will mimic the cell type and electrodes you will use. This is especially advisable if you are unfamiliar with the Axoclamp-2B, or with any recording mode that you may use.

All tutorials are written from the perspective that a computer coupled to an A/D and D/A interface is used to trigger the command pulses and monitor the current and voltage output of the Axoclamp-2B. Naturally, an oscilloscope and pulse generator can be used in place of the computer-based system. For the DCC and dSEVC modes an oscilloscope must be used to observe the MONITOR output because a rapid time base is required. A single oscilloscope with a dual time base could be used for recording the MONITOR output and the current and voltage outputs. Any source capable of generating timing or command pulses is suitable.

Summary of Controls, Inputs and Outputs

Please fold out the final page of the manual and refer to the figures of the front and rear panels of the instrument.

Mode Group

Illuminated pushbuttons reconfigure the Axoclamp-2B for different operating modes.

BRIDGE: Two conventional microelectrode amplifiers, ME1 and ME2.

DCC: Discontinuous current clamp on ME1; Bridge mode on ME2.

SEVC: Single-electrode voltage clamp on ME1; Bridge mode on ME2.

Discontinuous SEVC (dSEVC) uses time-sharing technique (electrode switches repetitively from voltage recording to current-passing).

Continuous SEVC (cSEVC) is analogous to whole-cell patch clamp (electrode simultaneously does voltage recording and current passing).

TEVC: Two-electrode voltage clamp. ME1 records voltage. ME2 passes current.

CONT./Discont**.:** The switch and lamp operate only in SEVC mode.

Microelectrode 1 (ME1) Group

This is a complete intracellular/extracellular electrometer.

CAPACITANCE NEUTRALIZATION: Neutralizes electrode input capacitance. Clockwise rotation reduces effective input capacitance and speeds response. Overutilization oscillates headstage.

BUZZ: Deliberate overutilization of capacitance neutralization. Oscillation helps cell penetration. Footswitches supplied as standard accessories can be used to actuate buzz. A buzz box, also supplied, controls the duration of the buzz.

BRIDGE: Compensates microelectrode voltage drop during current passing. Resistance (scaled by H) read on ten-turn dial. Range automatically reduced tenfold during cSEVC.

INPUT OFFSET: Adds ±500 mV DC to ME1 voltage at an early stage. Used to zero microelectrode tip potential while the microelectrode is extracellular.

DC CURRENT COMMAND: For injection of constant current. Magnitude set on ten-turn dial. Polarity set on switch. LED indicates when current injection activated.

CLEAR: Passes large hyperpolarizing or depolarizing current to clear blocked electrodes or facilitate cell impalement.

VOLTMETER: Indicates membrane potential (V_m) in mV.

Microelectrode 2 (ME2) Group

This is an independent intracellular/extracellular electrometer similar to ME1. It differs from ME1 in that the potential is labeled V_2 and OUTPUT OFFSET adds ±500 mV to the ME2 voltage at the output stage. Its recorded voltage can be read on the V_2 meter.

Voltage-Clamp Group

GAIN: Sets open-loop gain during voltage clamp. In SEVC modes the output is a current source. Therefore gain is nA/mV. In TEVC mode the output is a voltage source. Therefore gain is V/V.

HOLDING POSITION: Sets holding potential during voltage clamp. Range is ±200 mV.

RMP BALANCE LAMPS: Can be nulled using the HOLDING POSITION while in BRIDGE or DCC mode so that when the voltage clamp is activated, the voltage clamp will be at the resting membrane potential.

PHASE LAG: Modifies frequency response of voltage-clamp amplifier. Compensates for nonideal phase shifts of membrane. Potentiometer adds phase delay (lag). Switch selects range.

ANTI-ALIAS FILTER: Used in DCC or dSEVC modes to reduce noise of microelectrodes that have fast and slow settling characteristics.

Step-Command Group

Uses a D/A converter to generate a precise current or voltage command.

DESTINATION SWITCH: Selects the voltage clamp (VC) if in voltage clamp or either microelectrode (ME1 or ME2) if in a current clamp mode. Commands are mV or nA respectively.

THUMBWHEEL SWITCH: Sets magnitude with 0.05% resolution.

OFF/EXT./CONT. SWITCH: CONT. position activates STEP COMMAND. In the EXT. position the thumbwheel switch value is off unless logic level HIGH is applied to the rear-panel STEP ACTIVATE input. OFF position overrides logic input.

INDICATION: When destination is a microelectrode and STEP COMMAND is activated, lamp in microelectrode DC CURRENT COMMAND section illuminates.

Rate Group

A **Digital Counter** indicates the sampling rate (cycling rate) used during discontinuous single-electrode voltage clamp or discontinuous current clamp.

A potentiometer adjusts the rate from 500 Hz to 50 kHz.

Outputs

Two dedicated **Digital Voltmeters** continuously display the microelectrode voltages while a third displays the current in the selected microelectrode or in a virtual-ground circuit, if used. Front-panel rotary switches for each microelectrode and the virtual ground set the scaling of the current meter to suit the gain of your headstage.

In addition, an internally generated **Calibration Signal** can be superimposed onto each of the outputs. Hence, the output signals in many cases can be wholly conditioned within the Axoclamp-2B to suit your recording apparatus.

Five outputs are conveniently located at the front panel for connecting to your oscilloscope. These outputs are repeated at the rear panel, where the other outputs, the inputs and the headstage connectors are also located.

The 10 V_m, I_m OUTPUT BANDWIDTH switch selects the -3 dB frequency of two single-pole low-pass filters for the I_m and 10 V_m outputs.

The current (I) meter displays the DC current from either microelectrode or the virtual ground if used. A switch is used to select the meter input. The decimal point is set on H_1, H_2 or VG switches.

I_m OUTPUT: Membrane current recorded by ME1.

I_1 CONT. OUTPUT: ME1 current (equals I_m in Bridge, cSEVC and TEVC modes).

I_2 OUTPUT: ME2 current.

0.1 X I_2 OUTPUT: ME2 current; attenuated by ten.

I_{BATH} OUTPUT: Bath current.

10 V_m OUTPUT: Membrane potential recorded by ME1; gain of 10.

V_1 CONT. OUTPUT: Instantaneous ME1 potential. No Bridge Balance.

MONITOR OUTPUT: Input of sample-and-hold amplifier. Should be observed on second oscilloscope during DCC and dSEVC modes.

V_2 OUTPUT: ME2 potential. Includes Bridge Balance.

SAMPLE CLOCK OUTPUT: Logic-level pulses at the sample rate; used to trigger monitor oscilloscope.

V_{BATH} OUTPUT: Potential recorded by bath electrode.

Inputs

All inputs are located on the rear panel.

CAL. ACTIVATE INPUT: Logic HIGH on this input puts calibration voltage proportional to thumbwheel setting onto voltage and current outputs.

STEP ACTIVATE INPUT: Logic HIGH activates STEP COMMAND.

BLANK ACTIVATE INPUT: Logic HIGH activates Blank. During Blank, V_m prevented from updating. Thus stimulus artifacts are rejected.

EXT. VC COMMAND INPUT: Voltage on this input converted into voltage-clamp command.

EXT. ME1 COMMAND INPUT: Voltage on this input converted into ME1 CURRENT COMMAND.

EXT. ME2 COMMAND INPUT: Voltage on this input converted into ME2 CURRENT COMMAND.

R_S COMP. INPUT: Used to compensate voltage drop across membrane R_s during TEVC. Not normally required.

TBA: Spare connector (to be assigned).

Remote

Allows certain functions to be remotely activated by computer or switches. These are MODE, BUZZ and CLEAR.

Initial Instrument Settings (Default)

Before starting the tutorial for each mode, set the panel controls to the "default" settings indicated below. Note, **Minimum** values are fully counter-clockwise.

Step Command Group
STEP COMMAND Thumbwheel - **Zero**
EXT/CONT/OFF Switch - **Ext**
DESTINATION Switch - **ME1**

Microelectrode (ME1) and (ME2) Groups
CAPACITANCE NEUTRALIZATION - **Minimum**
BRIDGE - **Zero**
ME1: Input Offset - **None** (≈5)
ME2: Output Offset - **None** (≈5)
DC CURRENT COMMAND - **Switch OFF**

Rate Adjust
Minimum

I Display Select
I_m

Headstage Gain Selectors
H1 - 0.1
H2 - 1.0

V_m, I_m Output Bandwidth
30 kHz

Voltage Clamp Group
ANTI-ALIAS - **Minimum**
GAIN - **Minimum**
PHASE LAG - **0.01**
PHASE MULTIPLIER - **OFF**
HOLDING POSITION - **Any desired level**

Bridge Mode

Headstage Selection

This tutorial uses the HS-2A-x0.1LU and HS-2A-x1LU headstages shipped standard with the Axoclamp-2B. For other suitable headstages see *Headstages* in **REFERENCE GUIDE: PRINCIPLES OF OPERATION**.

Connections

Headstages

Connect the HS-2A-x0.1LU headstage to the ME1 PROBE connector and the HS-2A-x1LU headstage to the ME2 PROBE connector on the back panel of the Axoclamp-2B.

CLAMP-1U Model Cell

Switch the CLAMP-1U model cell to the BATH position. This simulates placing microelectrodes of 50 MΩ in the bath ready to impale a cell. Connect ME1 and ME2 of the CLAMP-1U model cell to corresponding headstages.

Connections to Interface and Signal Conditioner

To monitor the membrane voltage and current from ME1 connect the 10 V_m and I_m outputs to the inputs of your analog-to-digital acquisition system. The corresponding outputs for ME2 are V_2 and I_2. As the output filter applies only to the 10 V_m and I_m outputs, a second- or higher-order low-pass filter (*e.g.,* a CyberAmp 320) can be used to remove the high-frequency noise from 10 V_m.

Acquisition and Command Setup

Use one of the programmable logic outputs (TTLs) of your computer interface to synchronously apply a delayed logic pulse of 2 ms duration to the STEP ACTIVATE input on the rear of the Axoclamp-2B. The step command value on the thumbwheel will be directed to the circuit designated on the DESTINATION switch only when the toggle is switched to EXT. or CONT.

Alternatively, you could use D/A converters to send commands to the external ME1 and ME2 command inputs on the rear panel. Keep in mind that these inputs are simply summed with the commands generated by the internal command circuitry. The rear ME1 and ME2 current command inputs are continually active and are unaffected by the position of the command DESTINATION switch. For this reason check that "zero volts" of the command signal truly is zero volts, otherwise an offset current will appear through the electrode.

Balance the Bridge in the "Bath"

Turn the power on.

Now offset the voltage recorded on ME1 to zero using the INPUT OFFSET potentiometer. **Note:** Zero is at the middle of the dial range, very near 5.

Set a command current of 5.0 nA (although you can use a positive going pulse, negative pulses are an advantage with living cells) with the STEP COMMAND thumbwheel switch. Remember when setting the pulse magnitude that it is multiplied by the headstage gain (see ME1 on the DESTINATION switch). Thus, for an HS-2A-x0.1LU headstage, the correct STEP COMMAND setting is 50.

The I_m output can be used to display the current pulse. Since it is a square wave unchanged by the controls used in this tutorial, it is not shown.

Remember when observing the 10 V_m trace that the voltage output is multiplied by ten. Figure 1A shows the voltage response prior to adjusting the BRIDGE and the CAPACITANCE NEUTRALIZATION controls. Advance the BRIDGE dial until the fast voltage steps seen at the start and finish of the current step are just eliminated; the Bridge is then correctly balanced (Figure 1B). The model cell electrode resistance may now be read from the BRIDGE dial and should be 50 MΩ (sensitivity is $10 \div H$ MΩ per turn, where "H" is the headstage current gain, = 0.1 for the HS-2A-x0.1LU headstage).

The residual transient at the start and finish of the current step is due to the finite response speed of the microelectrode. No attempt is made to balance this transient since it covers a very brief period only and it is a useful indication of the frequency response of the microelectrode. Furthermore, no useful information during this period could be recovered even if the transient were balanced. The transient can be minimized by correctly setting the capacitance neutralization. Adjust the CAPACITANCE NEUTRALIZATION knob for the most rapid decay without causing an overshoot (Figure 1C). If the BRIDGE is over balanced the trace will look similar to that depicted in Figure 1D.

Use the corresponding controls of ME2 and the same procedure for the second microelectrode.

The BRIDGE controls operate on the 10 V_m output and on the V_2 output. On the 10 V_m output the BRIDGE control saturates when the IR voltage drop exceeds ±600 mV referred to the input.

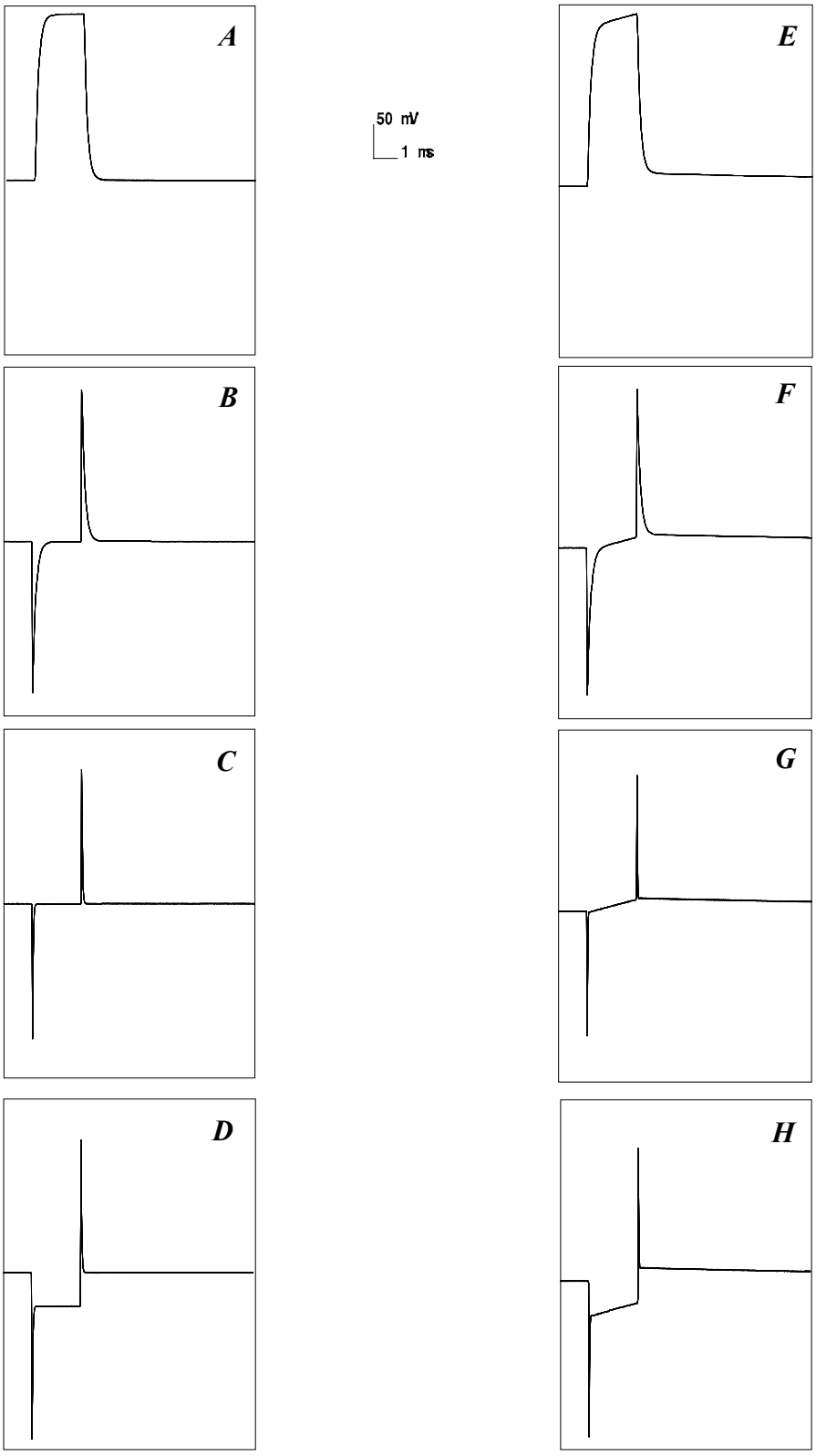

Figure 1. Bridge balancing procedure

Balance the Bridge in the "Cell"

When the microelectrode is in the cell any current flow through the microelectrode will produce an IR drop across the microelectrode that will add to the recorded membrane potential. The BRIDGE control can be used to balance this IR drop so that only the membrane potential is recorded.

Turn the CAPACITANCE NEUTRALIZATION and BRIDGE controls fully counterclockwise. Maintain the same connections and pulse parameters made above. Toggle the selector switch on the CLAMP-1U model cell to the CELL position. Prior to correctly setting the BRIDGE and CAPACITANCE NEUTRALIZATION controls, the voltage response will appear as in Figure 1E. The voltage responses appear more rounded than before due to the "cell membrane" time constant. Since the pulse width was fast compared with the membrane time constant, the membrane responses look like straight lines. The response was dominated by the IR voltage drop across the microelectrode.

When the BRIDGE is correctly balanced the trace will look like that depicted in Figure 1F. After the CAPACITANCE NEUTRALIZATION is set optimally, the trace will appear like that depicted in Figure 1G. If the BRIDGE is overused, the trace will look similar to that depicted in Figure 1H. It is possible that the CAPACITANCE NEUTRALIZATION setting found to be optimal during setup could be too large if the input capacitance were to decrease during the experiment. Therefore, it is suggested that capacitance neutralization be slightly underutilized.

The trace in Figure 2 was recorded in the CLAMP-1U model cell with the BRIDGE and CAPACITANCE NEUTRALIZATION controls set correctly. In response to a 40 ms positive current pulse the membrane potential began to charge up. Before the membrane potential reached its final value the current pulse was terminated and the membrane potential exponentially decayed to its final value.

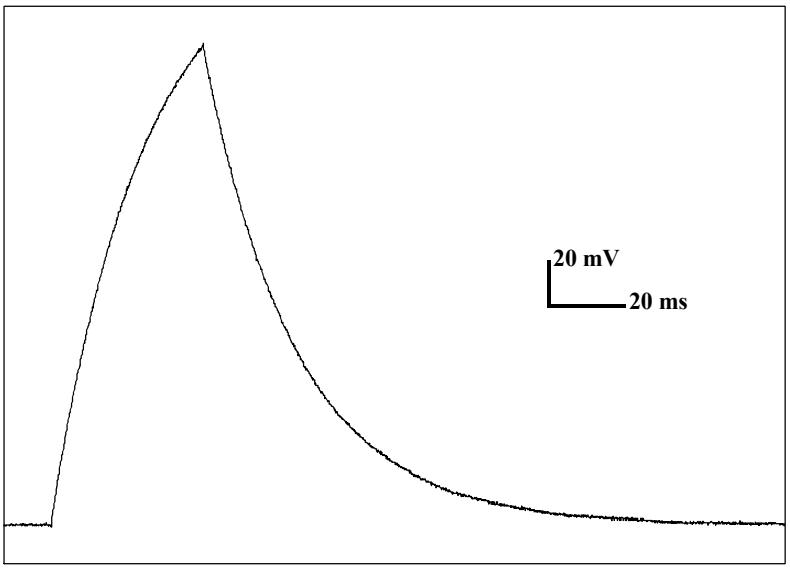

Figure 2. Correctly adjusted bridge and capacitance neutralization controls using the CLAMP-1U model cell

Continuous Single-Electrode Voltage Clamp (cSEVC) Mode

Headstage Selection

This tutorial uses the HS-2A-x0.1LU and HS-2A-x1LU headstages shipped standard with the Axoclamp-2B. For other suitable headstages see *Headstages* in **REFERENCE GUIDE: PRINCIPLES OF OPERATION**. Both headstages are used in the tutorial to illustrate the effects of properly adjusting the clamp. In an actual recording situation a single electrode is used.

Initial Instrument Settings

Before starting, set the panel controls to the "default" settings.

Connections

Headstages

Connect the HS-2A-x0.1LU headstage to the ME1 PROBE connector and the HS-2A-x1LU headstage to the ME2 PROBE connector on the back panel of the Axoclamp-2B.

Model Cell

Connect both headstages to the MCW-1U model cell (simulates the use of patch pipettes). To demonstrate the effect of series resistance compensation the second microelectrode, ME2, is used to record the true membrane potential.

Connections to Interface and Signal Conditioner

Monitor the ME1 output from 10 V_m and I_m. Monitor the ME2 output from V_2. Connect these outputs to a signal conditioner and low-pass filter the signals at 2 kHz to remove the high frequency noise. A second- or higher order low-pass filter (*e.g.,* a CyberAmp 320) can be used. Connect the output of the signal conditioner to the A/D interface.

Acquisition and Command Setup

Use one of the programmable logic outputs (TTLs) of your computer interface to synchronously apply a delayed logic pulse of 2.5 ms duration to the STEP ACTIVATE input on the rear of the Axoclamp-2B. The step command value on the thumbwheel will be directed to the circuit designated on the DESTINATION switch only when the toggle is switched to EXT.

> Alternatively, you could use D/A converters to send commands to the EXT. VC COMMAND input on the rear panel. Keep in mind that the input is simply summed with the commands generated by the internal command circuitry. The rear current command inputs are continually active and unaffected by the position of the command DESTINATION switch. For this reason check that "zero volts" of the command signal truly is zero volts, otherwise an offset current will appear through the microelectrode.

Balance the Bridge

Follow the steps outlined for balancing the BRIDGE and setting the CAPACITANCE NEUTRALIZATION.

Clamp the "Cell"

Use the HOLDING POSITION dial to yield equal brightness in each of the two RMP BALANCE LEDs. At this setting the command potential during voltage clamp will be equal to the resting membrane potential (RMP). Lock the HOLDING POSITION dial if desired.

Toggle to CONT. SEVC and press the yellow cSEVC MODE. Set up a repetitive step command and set the STEP COMMAND to 50 mV. Set the OFF\EXT.\CONT. switch to EXT. and set the DESTINATION switch to VC (mV).

For maximum stability switch the MULTIPLIER of the PHASE LAG to 10 or 100 and increase the PHASE LAG to 0.15. As the voltage clamp gain is increased, the PHASE LAG will also need to be readjusted. The GAIN and PHASE LAG will have to be iteratively adjusted to obtain the best response on both V_m and I_m. Sometimes lower current noise can be achieved for the same step response with less phase lag. Before switching to a lower phase lag setting reduce the voltage-clamp gain since the margin of stability is lower. Advance the BRIDGE dial to speed up the current and voltage settling times. Note that in cSEVC mode the BRIDGE dial implements series resistance compensation.

The Anti-Alias Filter is not recommended for use in cSEVC mode (but see *Anti-Alias Filter* in **REFERENCE GUIDE: PRINCIPLES OF OPERATION**).

An example of a cSEVC set up with a MCW-1U model cell is shown in Figure 3. All traces were low-pass filtered at 2 kHz using a CyberAmp 380. The voltage clamp gain was 17 nA/mV, the command was a 50 mV step and the phase lag was 1.2 ms. (Without phase lag it is possible to reduce the current noise by reducing the voltage clamp gain; however, as the gain is reduced, the capacitance transient takes longer to settle.) The ANTI-ALIAS FILTER was off and the CAPACITANCE NEUTRALIZATION setting was optimal. The figure consists of three parts with a series of three traces in each. The membrane current is shown in Figure 3A, the membrane potential plus the IR drop across the clamping microelectrode (ME1) are shown in Figure 3B and the true membrane potential recorded by the independent microelectrode (ME2) is shown in Figure 3C.

When no series resistance compensation is used (trace 1) there is a limit to how fast the membrane capacitance (Figure 3A) can be charged. This can be seen from the duration of the capacitance transient of the membrane current. Because the clamping microelectrode (ME1) records the true membrane potential as well as the IR drop across itself, the step response of the recorded voltage (Figure 3B) is faster than the true membrane potential (Figure 3C) recorded by the independent microelectrode (ME2). The time course of the true membrane potential is the same as that of the membrane current.

Trace 2 and trace 3 show the effect of 35% and 70% series resistance compensation, respectively. Note the substantial improvement in speed of both the current (Figure 3A) and true membrane potential (Figure 3C).

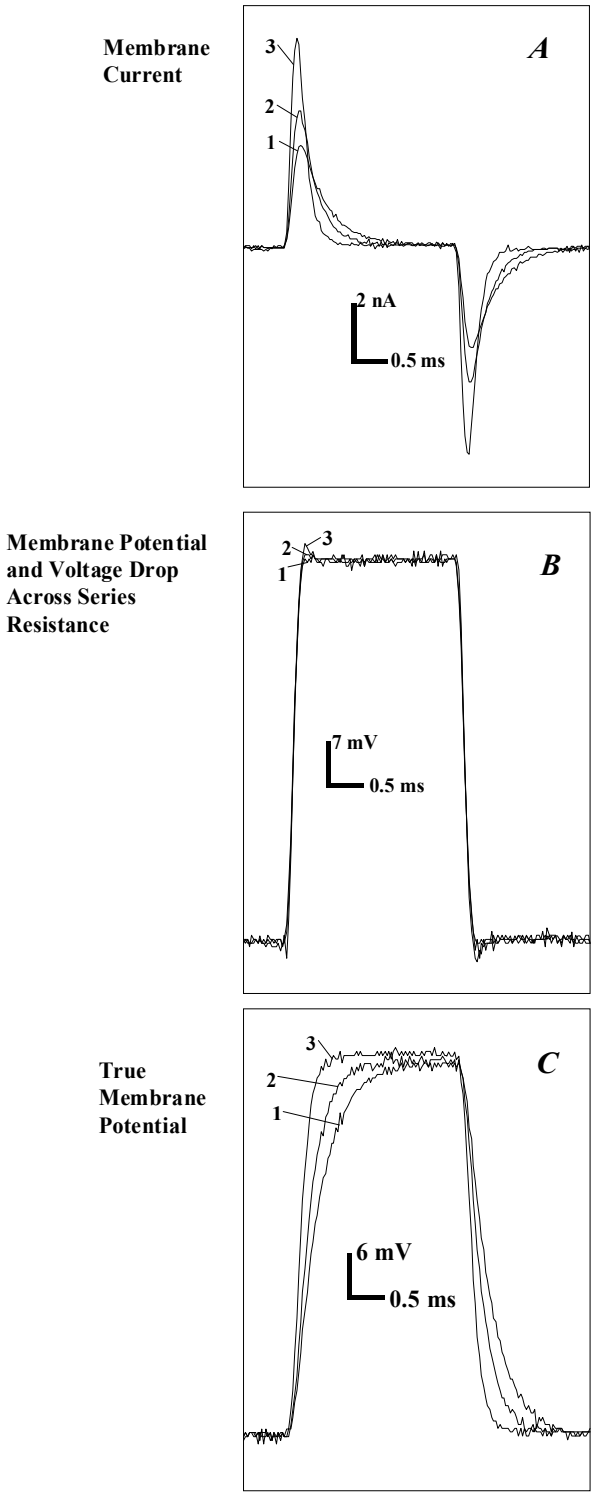

Figure 3. Current and potential recording during cSEVC in a cell model

When recording from a real cell you will not be able to use the true membrane potential to determine the optimal series resistance setting. Instead, the current trace can be monitored while adjusting the clamp settings.

Discontinuous Current Clamp Mode - DCC

Headstage Selection

This tutorial uses the HS-2A-x0.1LU headstage shipped standard with the Axoclamp-2B. For other suitable headstages see *Headstages* in **REFERENCE GUIDE: PRINCIPLES OF OPERATION**.

Initial Instrument Settings

Before starting, set the panel controls to the "default" settings.

Connections

Headstages

Connect the HS-2A-x0.1LU headstage to the ME1 PROBE connector on the back panel of the Axoclamp-2B.

Model Cell

Connect the headstage to ME1 of the CLAMP-1U model cell (the MCW-1U model cell can be used if you wish to simulate the use of a patch pipette). Make sure the CLAMP-1U model cell is in the CELL position.

Connections to Interface and Signal Conditioner

Monitor the ME1 output from 10 V_m and I_m. Connect these outputs to the A/D interface.

Connections to the Oscilloscope

Connect the MONITOR output to one of the input channels of the monitor oscilloscope (which need not be a high quality type) with the gain at 100 mV/div (= 10 mV/div input referred). The output of the SAMPLE CLOCK is used to trigger the oscilloscope.

Acquisition and Command Setup

Use one of the programmable logic outputs (TTLs) of your computer interface to synchronously apply a delayed logic pulse of 10 ms duration to the STEP ACTIVATE input on the rear of the Axoclamp-2B. The step command value on the thumbwheel will be directed to the circuit designated on the DESTINATION switch only when the toggle is switched to EXT.

> Alternatively, you could use D/A converters to send commands to the EXT. ME1 COMMAND input on the rear. Keep in mind that the input is simply summed with the commands generated by the internal command circuitry. The rear ME1 current command input is continually active and is unaffected by the position of the command DESTINATION switch. For this reason check that "zero volts" of the command signal truly is zero volts, otherwise an offset current will appear through the microelectrode.

Make sure the ANTI-ALIAS FILTER is set to the minimum value and switch to DCC mode. Set the DESTINATION switch to ME1 and set up a repetitive square current command of 3 nA.

Adjust the Sample Rate and the Capacitance Neutralization

Switch the OFF/EXT./CONT. switch to CONT., continuous. Switch to DCC mode by pressing the yellow button. Keep the pulse amplitude used above. Observe the voltage at the ME1 headstage on the second oscilloscope which is triggered from the SAMPLE CLOCK output. The sweep frequency should be set to 20 µs/div. At this stage, set the sample rate to about 8.0 kHz (selecting the sample rate is discussed in *DCC or dSEVC* in **REFERENCE GUIDE: THEORY OF RECORDING MODES**) using the RATE ADJUST knob.

Set the OUTPUT BANDWIDTH to 1/5 or 1/10 of f_s. Set the filter to 1 kHz for this tutorial. If you wish to use an external filtering device to filter the 10 V_m and I_m, remember to set the bandwidth of the Axoclamp-2B to 30 kHz.

When using the discontinuous current or voltage clamp mode, it is essential to obtain optimal capacitance neutralization. For optimum capacitance neutralization, advance the CAPACITANCE NEUTRALIZATION control until the MONITOR waveform decays most rapidly to a horizontal baseline without any overshoot or undershoot.

Reduce the noise on the 10 V_m and I_m traces either by advancing the ANTI-ALIAS FILTER or by increasing f_s, adjusting the capacitance neutralization where necessary.

An alternative method can yield acceptable, but not optimal, capacitance neutralization. Apply a repetitive current pulse, then advance the CAPACITANCE NEUTRALIZATION control until the square step at the leading edge of the 10 V_m response is first eliminated. If the square step cannot be eliminated (without overshoot on the MONITOR waveform), decrease the sample rate (f_s).

The two methods for adjusting capacitance neutralization during *DCC* are illustrated in Figure 4. All traces were recorded using a CLAMP-1U model cell. The cycling rate was 8.0 kHz and the current pulse was 30 nA with a 10 ms duration. The ANTI-ALIAS FILTER was set to 9 µs. The outputs from 10 V_m and MONITOR are shown in Figure 4A and B, respectively. There are three pairs of corresponding traces.

Trace 1 is an example of underutilization of capacitance neutralization. There is a fast step in 10 V_m (Figure 4A) at the start and finish of the current pulse because the MONITOR waveform (Figure 4B) decayed too slowly to reach its final value.

Optimal capacitance neutralization is shown in Trace 2. The 10 V_m trace (Figure 4A) shows the membrane response only. The MONITOR waveform decay (Figure 4B) is fast with no overshoot and easily reaches the final value.

Capacitance neutralization is overutilized in Trace 3. The fast steps in V_m (Figure 4A) reappeared, this time because of overshoot in the MONITOR waveform (Figure 4B). Note that unlike a bridge circuit, the effect of too much compensation can put either a positive or a negative step on V_m (positive in this example) depending on which cycle of the ringing in the MONITOR waveform is sampled.

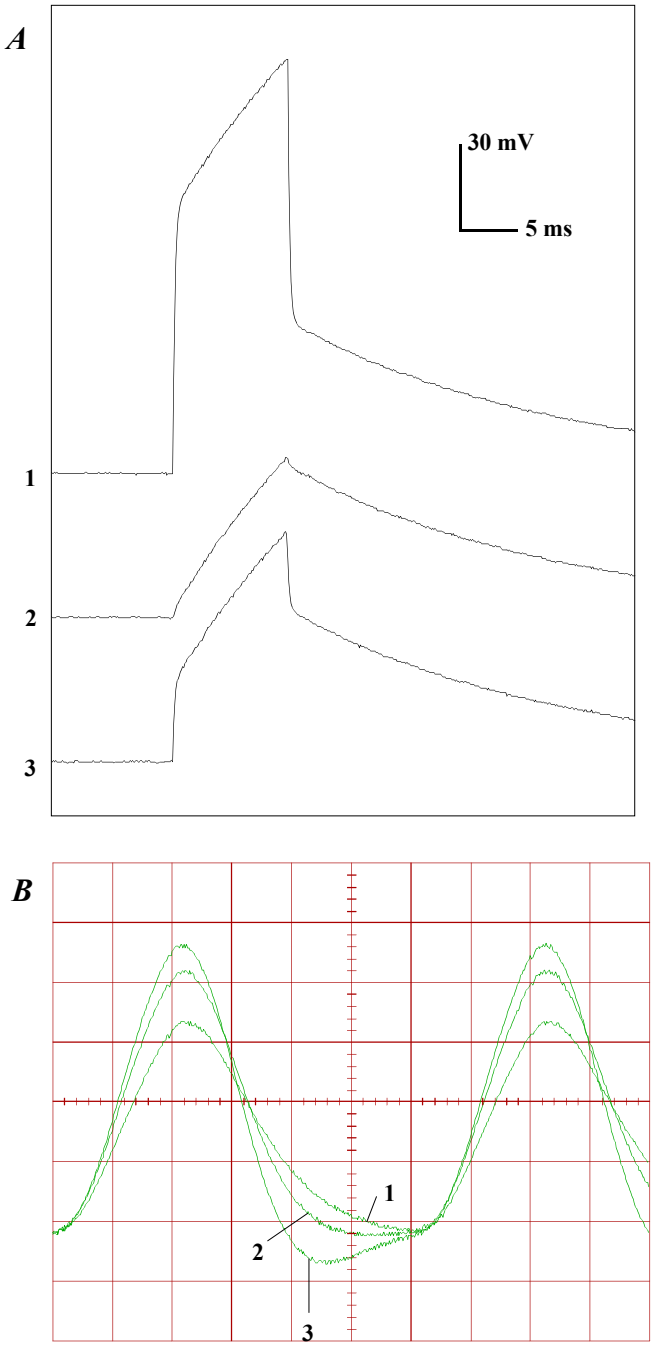

Figure 4. How to set the capacitance neutralization during DCC mode

Discontinuous Single-Electrode Voltage Clamp Mode - dSEVC

Headstage Selection

This tutorial uses the HS-2A-x0.1LU and HS-2A-x1LU headstages shipped standard with the Axoclamp-2B. For other suitable headstages see *Headstages* in **REFERENCE GUIDE: PRINCIPLES OF OPERATION**. Both headstages are used in the tutorial to illustrate the effects of properly adjusting the voltage clamp. In an actual recording situation a single microelectrode is used.

Initial Instrument Settings

Before starting, set the panel controls to the "default" settings.

Connections

Headstages

Connect the HS-2A-x0.1LU headstage to the ME1 PROBE connector and the HS-2A-x1LU headstage to the ME2 PROBE connector on the back panel of the Axoclamp-2B.

Model Cell

Connect both headstages to the CLAMP-1U model cell (the MCW-1U model cell can be used if you wish to simulate the use of a patch pipette). The HS-2A-x1.0LU records the true membrane potential and is used as part of the demonstrations indicating the correct and false settings of the clamp.

Connections to interface

Monitor the ME1 output from 10 V_m and I_m. Monitor the ME2 output from V_2. The second microelectrode, ME2 is used to measure the true membrane potential. Connect the outputs to the A/D interface. Although a signal conditioner is not needed for this tutorial, it is useful for filtering the output of V_2. A second- or higher order low-pass filter (*e.g.,* a CyberAmp 320) can be used.

Connections to the Oscilloscope

Connect the MONITOR output to one of the input channels of the monitor oscilloscope (which need not be a high quality type) with the gain at 100 mV/div (= 10 mV/div input referred). The output of the SAMPLE CLOCK is used to trigger the oscilloscope.

Acquisition and Command Setup

Use one of the programmable logic outputs (TTLs) of your computer interface to synchronously apply a delayed logic pulse of 2.5 ms duration to the STEP ACTIVATE input on the rear of the Axoclamp-2B. The step command value on the thumbwheel will be directed to the circuit designated on the DESTINATION switch only when the toggle is switched to EXT.

> Alternatively, you could use D/A converters to send commands to the EXT. VC COMMAND input on the rear panel. Keep in mind that the input is simply summed with the commands generated by the internal command circuitry. The rear current command inputs are continually active and are unaffected by the position of the command DESTINATION switch. For this reason check that "zero volts" of the command signal truly is zero volts, otherwise an offset current will appear through the microelectrode.

Adjust the Sample Rate and Capacity Neutralization

Before switching into dSEVC mode, set up in DCC mode. Follow the steps outlined in the DCC mode for adjusting the sampling rate and setting the capacitance neutralization with the CLAMP-1U model cell switched to the CELL position. If you wish, filter the output from V_2 with an external filtering device.

Clamp the "Cell"

Turn the OFF/EXT/CONT. switch to OFF. Press the green BRIDGE mode button to record membrane potential (which in this case is zero). Use the HOLDING POSITION dial to achieve equal brightness in the RMP BALANCE LEDs. When you switch to clamp mode the membrane will automatically be clamped at the resting membrane potential of the model cell, *i.e.,* 0 mV. Naturally, in a living cell the resting membrane potential will be some hyperpolarized value.

Set up a repetitive command pulse. Make this pulse 60 ms in duration and trigger the pulse every second. Switch the OFF/EXT./CONT. switch to EXT. and the DESTINATION switch to VC.

Toggle to DISCONT. SEVC and press the red SEVC button. Increase the voltage clamp GAIN control as far as possible without causing overshoot or instability in the step response. Reduce the GAIN slightly below the maximum value to get a safety margin. This will sharpen both the voltage and current responses.

Increase the ANTI-ALIAS FILTER while checking the settling characteristics of the monitor waveform. The noise on 10 V_m and I_m may be reduced by this procedure. Only use as much anti-alias as is consistent with stability.

Note: It is sometimes necessary to iteratively re-adjust the ANTI-ALIAS FILTER and CAPACITANCE NEUTRALIZATION controls to achieve the best clamp conditions.

An example of a correctly set up dSEVC is shown in Figure 5. Gain was 0.7 nA/mV and the sampling rate was 8 kHz. No phase lag was used. Capacitance neutralization was optimum.

Traces displayed in Figure 5 are the membrane current, I_m (Figure 5A), the sample membrane potential, V_m (Figure 5B) and the true membrane potential, V_2 (Figure 5C) recorded by an independent microelectrode. These outputs of the Axoclamp-2B were low-pass filtered at 2 kHz using a CyberAmp 380. Note that the two voltage records are identical because the capacitance neutralization was correctly set.

In Figure 5D, multiple sweeps of the MONITOR waveform are shown. This record was taken with the cell held at rest. The current pulses vary from sweep to sweep because of the sampled voltage noise. The important feature is that the voltage transients decay completely by the time the samples are taken (arrow) even for the largest transients.

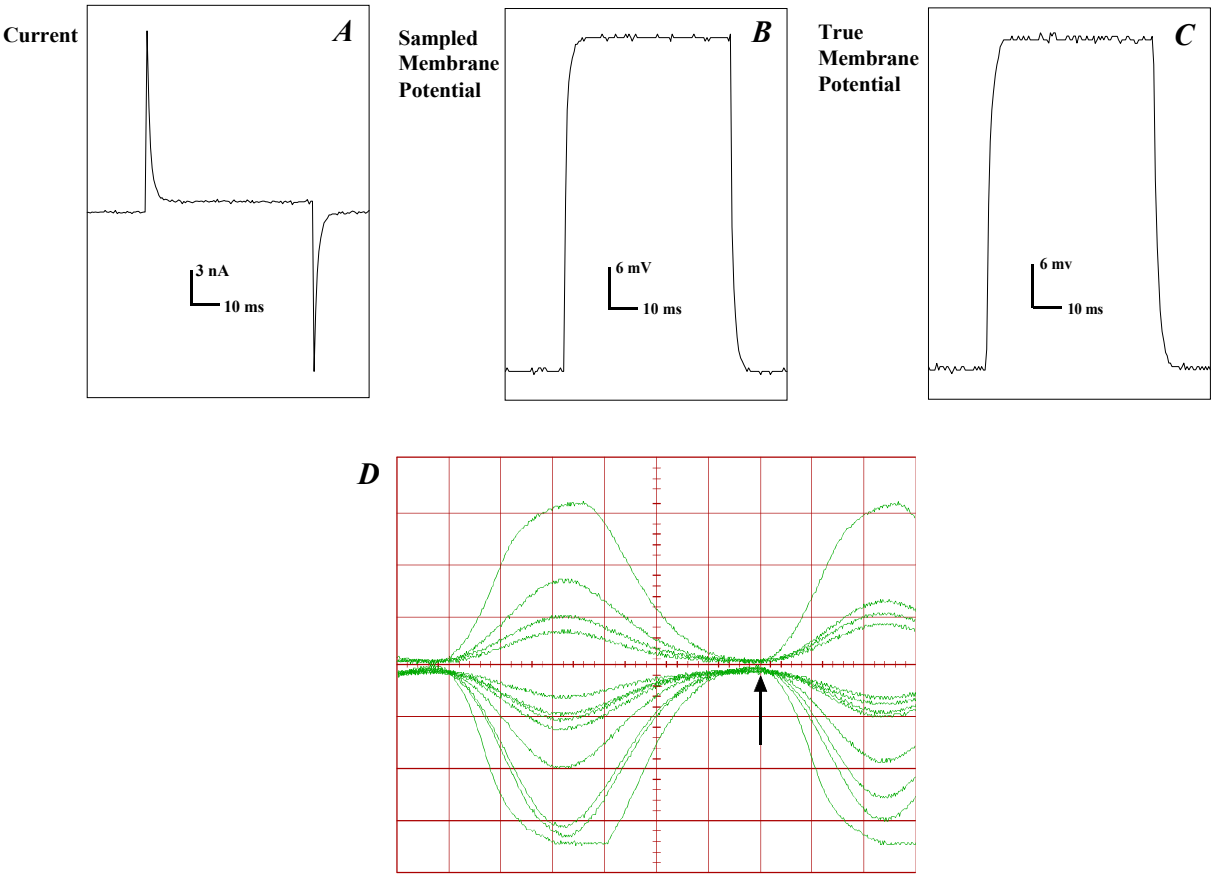

Figure 5. Correctly set up dSEVC in a cell model

False Clamp

If the PHASE LAG controls are used it is possible to find false settings of CAPACITANCE NEUTRALIZATION (or the ANTI-ALIAS FILTER) and PHASE LAG which together give a seemingly fast step response to V_m whereas in fact the step response in the cell is much slower.

This situation arises by underutilizing the CAPACITANCE NEUTRALIZATION (or overutilizing the ANTI-ALIAS FILTER) control so that the MONITOR waveform fails to decay adequately when the voltage sample is taken. The sampled microelectrode voltage has the nature of an IR drop across a series resistance (R_s; see *Series Resistance* section). Normally this would make the clamp unstable, but by introducing phase lag, stability can be re-imparted although without any reduction of the voltage error.

This false condition only arises if the CAPACITANCE NEUTRALIZATION setting is altered after the PHASE LAG control has been switched in. There are two ways to guarantee that this "false clamp" will not occur.

1) Do not use the PHASE LAG.
2) If the PHASE LAG is used be sure to conscientiously observe the MONITOR waveform to make sure that the decay to a horizontal baseline is complete at the end of each cycle.

An example of a false clamp is shown in Figure 6. The recorded value of I_m is always a true measure of the membrane current even during this false setting. Only the V_m record is erroneous. The danger of this false condition is that most of the presumed membrane potential is in fact voltage drop across the microelectrode.

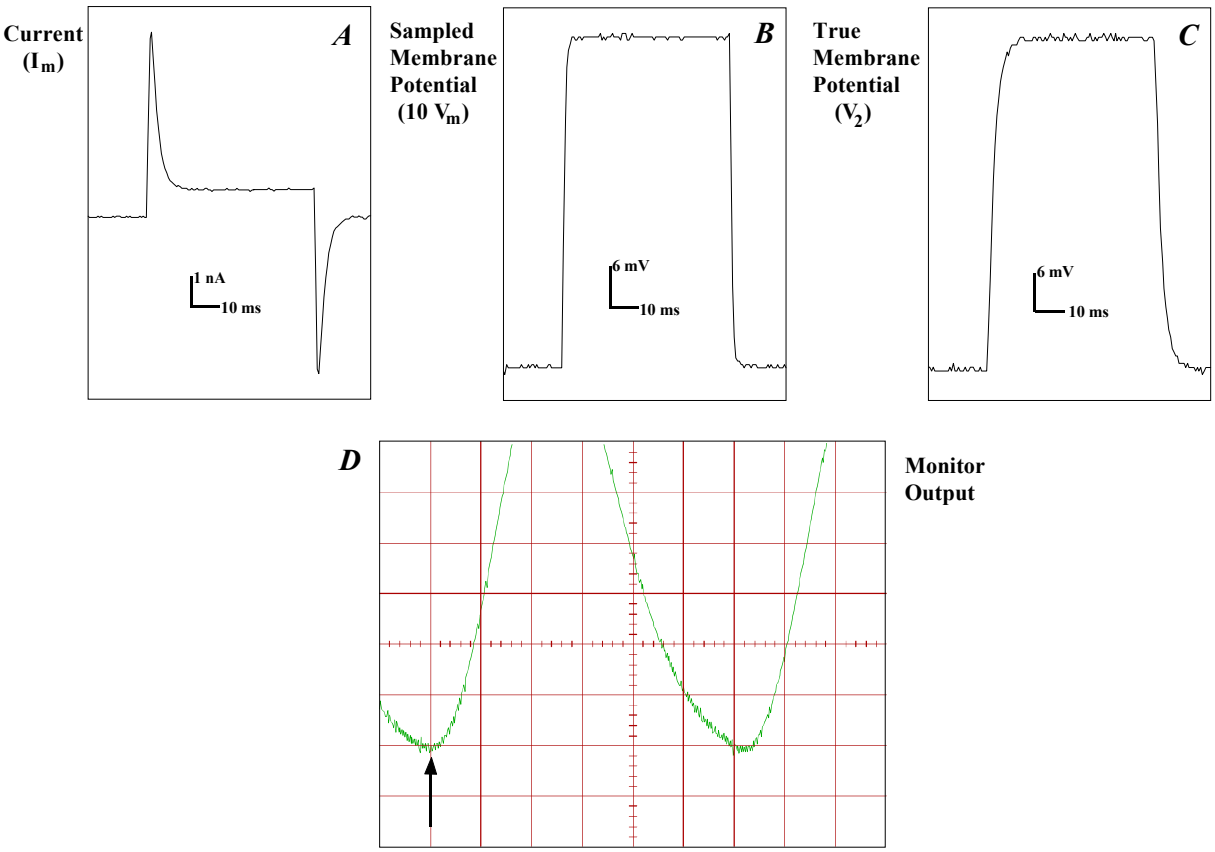

Figure 6. Incorrectly set up dSEVC (*i.e.,* "False" clamp) in a cell model

The cell model, R_{e1}, headstages and settings of the Axoclamp-2B were the same as in Figure 5. However, phase lag was 10 ms and capacitance neutralization was under-utilized. Membrane current, Figure 6A, is much smaller and slower than the one in Figure 5A.

Sampled membrane potential (available at the 10 V_m output) and true membrane potential recorded by an independent microelectrode are displayed in Figure 6B and Figure 6C, respectively. Notice that the time courses of the two voltage records are not the same. The sampled membrane potential includes a large error due to the voltage across the microelectrode at the sampling time (Figure 6D above).

Figure 6D shows the MONITOR waveform. It was recorded with the cell held at +50 mV from rest. (This was done because when the cell was held at rest with the considerable amount of phase lag used the noise current pulses were too small to allow the adequacy of the decay to be seen.) The voltage transient did not decay to a horizontal baseline at the time the sample was taken (arrow), therefore the sample included some of the IR voltage drop across the microelectrode.

TEVC MODE

Headstage Selection

This tutorial uses the HS-2A-x0.1LU and HS-2A-x1LU headstages shipped standard with the Axoclamp-2B. For other suitable headstage combinations see *Headstages* in **REFERENCE GUIDE: PRINCIPLES OF OPERATION**.

Initial Instrument Settings

Before starting, set the panel controls to the "default" settings.

Connections

Headstages

Connect the HS-2A-x0.1LU headstage to the ME1 PROBE connector and the HS-2A-x1LU headstage to the ME2 PROBE connector on the back panel of the Axoclamp-2B.

Model Cell

Switch the CLAMP-1U model cell to the BATH position. This simulates placing the microelectrodes in the bath ready to impale a cell. Connect ME1 and ME2 of the CLAMP-1U model cell to the corresponding headstages (the MCW-1U model cell can be used if you wish to simulate the use of patch pipettes).

Connections to Interface and Signal Conditioner

Connect the 10 V_m, V_2 and I_2 (or I_{BATH}) outputs to the inputs of the A/D interface. If large currents are to be passed, use the 0.1 x I_2 output to attenuate the magnitude of the current signal so as not to exceed the ±10 V range of the interface. A second- or higher order low-pass filter (*e.g.,* a CyberAmp 320) can be used to remove the high-frequency noise from I_2.

Optional Connections to an Oscilloscope

The error in the clamped membrane potential can be used as an indication that there is a problem with the clamp. To monitor the error use an oscilloscope with two input channels. First ground the two channels and offset the DC levels to zero. Set the 10 V_m,I_m OUTPUT BANDWIDTH of the Axoclamp-2B to 10 kHz. Connect the 10 V_m output to one input. Use a BNC "T" piece to connect the EXT. VC COMMAND signal to the other input. Use a TTL output from the A/D interface to trigger the oscilloscope.

Note: You will have to set the sensitivity of the 10 V_m channel to be five-fold greater than the EXT. VC COMMAND channel, since the EXT. VC COMMAND signal is larger than the true command value by 50 fold. If the voltage clamp is operating accurately then there should be very little, if any, observable difference (*i.e.,* error) between V_m and the EXT. VC COMMAND. Note that you may see a transient difference in the two traces at the onset of the step, since the rise time of V_m will not be as fast as the rise time of the EXT. VC COMMAND.

Acquisition and Command Setup

Use one of the programmable logic outputs (TTLs) of your computer interface to synchronously apply a delayed logic pulse of 6 ms duration to the STEP ACTIVATE input on the rear of the Axoclamp-2B. The step command value on the thumbwheel will be directed to the circuit designated on the DESTINATION switch only when the toggle is switched to EXT.

> Alternatively, you could use D/A converters to send commands to the EXT. VC COMMAND input on the rear panel. Keep in mind that these inputs are simply summed with the commands generated by the internal command circuitry. The rear current command inputs are continually active and are unaffected by the position of the command DESTINATION switch. For this reason check that "zero volts" of the command signal truly is zero volts, otherwise an offset current will appear through the microelectrode.

Balance the Bridge

Follow the procedure outlined in the Bridge Mode tutorial to set the capacitance neutralization of each microelectrode for the best step responses.

The switch that selects the BATH and CELL modes of the model cell reduces the capacitance coupling between the electrodes. When recording from a real cell a grounded shield is **required** (see *TEVC* in **REFERENCE GUIDE: THEORY OF CLAMP MODES**).

After correctly setting the BRIDGE and CAPACITANCE NEUTRALIZATION controls, switch the CLAMP-1U model cell to the CELL position to simulate a cell impaled by the micropipettes. The voltage responses will now appear more rounded than before due to the "cell membrane" time constant. The pulse duration may have to be increased to allow the voltage responses to reach steady state. Measure the amplitude of the responses and calculate the cell input resistance.

Tune the Voltage Clamp

Use the HOLDING POSITION control to yield equal brightness in each of the two RMP BALANCE LEDs. At this setting the command potential during voltage clamp will be equal to the resting membrane potential (RMP). Lock the HOLDING POSITION control if desired. In a real cell, setting the holding level to the cell resting potential can be done by adjusting the HOLDING POSITION dial until the two LEDs are equally dim. Do this before turning on the command pulses.

Ensure that the voltage clamp gain is at a minimum and there is no phase lag. The ANTI-ALIAS FILTER slows the ME1 electrode response and is not used in TEVC mode; set it at the minimum (see *Anti-Alias Filter* in **REFERENCE GUIDE: PRINCIPLES OF OPERATION**).

Turn on the voltage clamp by pressing the blue TEVC button. Start the step command and set the thumbwheel switch to 50 mV. To obtain the best step response (the fastest possible step without significant oscillation) the voltage-clamp gain setting must be high enough to guarantee that the voltage clamp tracks the command potential accurately even during activation of large membrane currents. A rough calculation of the minimum tolerable gain can be made from the equations given in the *Series Resistance* section of the **REFERENCE GUIDE: PRINCIPLES OF OPERATION** chapter.

With the GAIN control at its minimum value, the voltage trace should appear rounded. Slowly increase the GAIN setting and notice that the voltage trace rises much faster and the capacitive transient of the current trace becomes much sharper and decays more rapidly to baseline. Eventually a point will be reached when increasing the voltage clamp gain will result in oscillations. Reduce the gain so there are no oscillations. The voltage clamp is tuned properly if there are no oscillations and

the voltage trace is maximally square. Concurrently, the current trace peak sharpens and its rate of return to baseline is most rapid.

Figure 7 shows the current and voltage traces obtained while tuning the clamp using the CLAMP-1U model cell. The current and voltage traces are shown in parts A and B, respectively. Trace 1 represents the condition in which the GAIN setting is 150 V/V. As the gain is increased to 300 V/V (Trace 2) the voltage trace becomes more square, the current trace sharpens and its decay to baseline becomes much more rapid. At a gain of 600 V/V, the voltage clamp is optimally tuned (Trace 3).

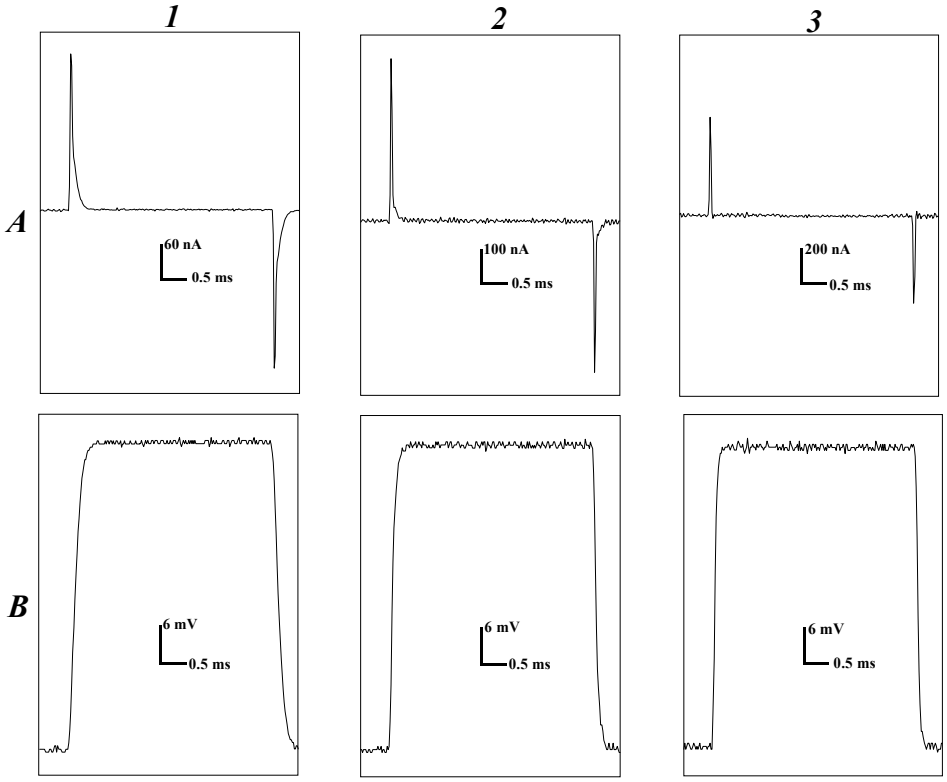

Figure 7. Tuning the TEVC with the CLAMP-1U model cell

If you are using an oscilloscope, monitor the onset of the step response in detail by turning the oscilloscope sweep to 0.1-0.2 ms/div. Slowly turn up the gain, and observe the voltage step become larger and more square. Eventually a point is reached where V_m overshoots the step value. Reduce the sweep speed (1 ms/div) and increase the voltage clamp gain a little more. You will see clearly that V_m displays damped oscillation during a voltage step. The oscillation in V_m will gradually die away until V_m stabilizes at the step potential. The damping time-constant depends on the gain. Increase the gain further (with a real cell, it may not be possible to further increase the gain) and the oscillations will take longer to fade until at even higher gains the clamp will oscillate continually. If this were a real cell the membrane would almost certainly have been destroyed.

Generally the capacitance neutralization level for each microelectrode is set in BRIDGE mode and then left. However, adjusting the capacitance neutralization of ME1 in TEVC mode will have a significant effect on the speed of the step response. This is to be expected, since the voltage clamp cannot operate faster than ME1. In fact, reducing the capacitance neutralization level is like adding phase lag and over compensating is like adding phase lead. Even so, using capacitance neutralization

for this purpose is not recommended, since changes in the solution level of the chamber can have significant effects on C_{in} which could in turn lead to unexpected and potentially disastrous effects on the stability of the voltage clamp. It is better to slightly under-compensate C_{in} and rely on the built in phase compensation circuitry. The capacitance neutralization of ME2 is not so critical as ME1, and minor changes in this control under voltage clamp can be used to make slight improvements to the step response.

The effect of phase lag can be demonstrated using the optional **MCO-1U** model cell. Connect the HS-2A-x1LU (in the ME1 position) and HS-2A-x10MGU headstage (in the ME2 position) to the indicated parts of the model cell. Insert one pin of the four-leaded connector into the gold case ground (brass socket); another pin into the white BATH socket ground and the third pin into the rear of the HS-2A-x1LU headstage. Connect the clip lead to the shield. Use the 0.1 x I_2 BNC to monitor the current output.

Figure 8 illustrates the effects of phase lag on the current. As the voltage clamp gain is slowly increased the current response will begin to sharpen. If the gain is further increased to 10,000 with the PHASE LAG control set to 0.5 ms, the voltage clamp becomes unstable. This is indicated by oscillations on both the current (Figure 8A) and voltage (not shown) records. Oscillations are to be avoided when recording from real cells because the cell membrane is severely damaged. In cells whose membranes do not cause the same phase shift (90°) as a parallel RC cell model, the PHASE LAG control can be used to increase the maximum gain achievable. To improve stability it is simply a matter of empirically finding the settings that work best for your particular system. With the **MCO-1U** model it is possible to achieve a stable voltage clamp with a GAIN setting of 10,000 V/V, once the PHASE LAG is increased to 0.15 ms (Figure 8B).

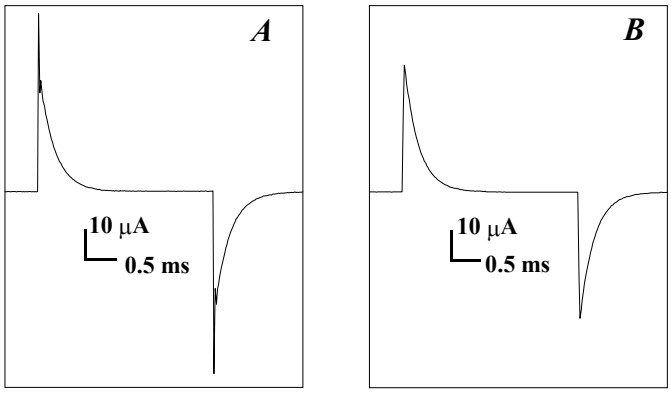

Figure 8. Tuning the TEVC with the **MCO-1U** model cell

MICROELECTRODES

Microelectrodes for Fast Settling

The key to discontinuous voltage and current clamping with a single microelectrode is the character of the microelectrode itself. The microelectrode voltage must settle rapidly after a current pulse, and the microelectrode must be able to pass current without large changes in resistance.

To maximize the performance of the two-electrode voltage clamp the high frequency performance of the voltage recording microelectrode must be preserved. Likewise in the continuous single-electrode voltage clamp mode the performance of the clamp is governed by the patch pipette.

The important factors that need to be considered are given below.

Microelectrode Capacitance

To achieve fast settling it is essential to minimize the transmural capacitance (C_t) from the inside of the microelectrode to the external solution. C_t is usually 1-2 pF per mm of immersion. Two applications requiring different approaches are discussed here.

Target Cell Near Surface of Solution

In an isolated preparation, C_t can be reduced by lowering the surface of the solution as far as possible. For a long slender microelectrode we regard 200 µm or less as a low solution level; 500 µm is tolerable. Deep is regarded as 1 mm or more. For a microelectrode which tapers steeply (*i.e.,* a stubby microelectrode) deeper solutions can be used with less loss of performance. When working with very low solution levels there is a risk of evaporation exposing the cells to the air unless a continuous flow of solution is provided across or through the preparation. If evaporation is a problem one way to overcome it is to float a layer of mineral oil on the surface of the solution. If used, this layer of oil will have the additional advantage of automatically coating the microelectrode as it is lowered into the solution.

Precautions must be taken to prevent surface tension effects from drawing a thin layer of solution up the outer wall of the microelectrode. If this film of saline is allowed to develop, C_t will be much worse that otherwise. Because the film of saline has axial resistance the contribution to C_t will be very nonlinear, and the voltage decay after a current pulse will either be biphasic (as in Figure 9), or if it is monophasic it will not be very fast even when capacitance neutralization is used. To prevent the saline film from developing, the microelectrode should be coated with a hydrophobic material. This can be done just before use by dipping the **filled** microelectrode into a fluid such as silicone oil or mineral oil. Another method is to coat the microelectrode with Sylgard (Dow Corning, Midland, MI).

Sylgard or Q-dope (airplane glue) can also be used to build up the wall thickness of the microelectrode, thereby reducing C_t. The selected material should be painted onto the electrode to within 100 µm of the tip.

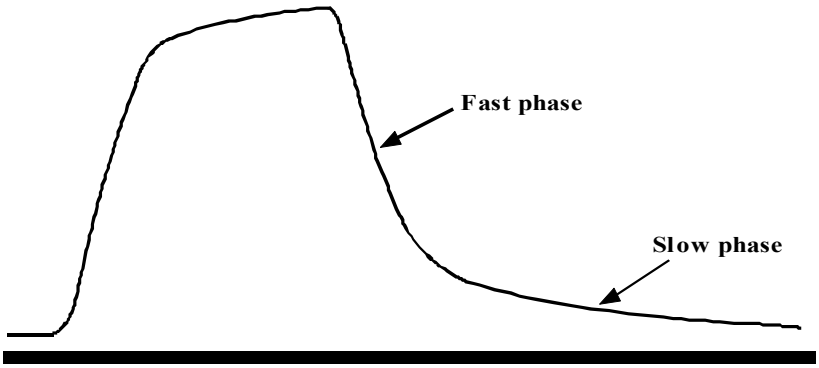

Figure 9. Two-phase microelectrode decay

Target Cell Deep in Solution

In some preparations, *e.g., in vivo* CNS, the target cell is several millimeters below the surface of the solution. In this case the more difficult procedure of guarding the microelectrodes may have to be used. This involves coating the outside of the microelectrode with a metal layer and connecting this layer to the case socket of the unity-gain headstage. Depending upon the headstage gain, the case socket is either connected to the capacitance neutralization circuit or to the unity-gain output (see Shield Drive Connector on p. 73). The guarding procedure does not reduce C_t. Instead, it reduces the **effect** of C_t by controlling the voltage across it. The metal guard layer must be insulated from the preparation solution. For different approaches to this method see Schwartz & House (1970), Suzuki, Rohliçek & Fròmter (1978), Sachs & McGarrigle (1980) and Finkel & Redman (1983).

Shielding the microelectrode introduces high-frequency noise; therefore it should only be done when absolutely necessary. The amount of added noise is proportional to the amount of shield capacitance, so only the minimum necessary length of microelectrode should be shielded.

Because of the distributed nature of the axial resistance of the microelectrode, of the axial resistance of the metal layer, and of C_t, the shielding technique is not perfect. In practice, the effect of these nonidealities is to cause the step response of the microelectrode to overshoot even when the capacitance neutralization gain is unity. For this reason, the capacitance neutralization circuit has a minimum less than unity.

Capacity Transients and Solution Levels

If it is important for your application to obtain very accurate subtraction of the linear capacitive currents seen during step changes in voltage, then the bath solution level must be as stable as possible. The reason for this is that changes in the solution level can have significant effects on the stray capacitance of the current-passing microelectrode. Since this capacitance must be charged by the voltage-clamp output, changes in the solution levels can lead to erroneous (though small) changes in the linear component of the capacitive transients. When using the virtual ground technique the problem is potentially worse since the solution level on both the current-passing microelectrode and virtual ground electrode changes and affects their coupling capacitance. Again this will lead to erroneous changes in the linear capacitive currents.

Micropipette or Patch Pipette?

The type and resistance of the microelectrodes will depend on the particular application and ultimately personal preference, but there are a few points that should be considered.

Patch pipettes offer some advantages over intracellular micropipettes. First, the recording configuration is far more mechanically stable. Second, stable recordings can be obtained with patch pipette resistances one to two orders of magnitude lower than those of micropipettes.

This second point is most important and a number of benefits accrue. Due to its low resistance a patch pipette used for voltage recording will have a better frequency response and lower noise level than a micropipette, resulting in a voltage-clamp system with much lower noise and superior fidelity and dynamic response. The tip potential of high resistance intracellular micropipettes is often unstable and can change erratically as the cell is penetrated. The tip potential of patch pipettes is stable and can be accurately measured and corrected for. Low resistance patch pipettes can more easily pass the large currents required to clamp the cell during conductance changes and step changes in the command voltage. In contrast, during large current transients a high resistance micropipette can become highly non-linear, due to ionic depletion effects at the tip.

There are some instances where micropipettes may be more useful. If your study requires that the contents of the cell remain relatively intact (second messenger systems for example), then patch pipettes may not be appropriate since the diffusible cellular components will eventually become diluted. In such cases the user may also wish to consider the "perforated patch" technique which prevents the loss of large intracellular molecules to the patch pipette (see *Patch* section in **REFERENCES**).

Micropipettes

Electrode Glass

Borosilicate glass is often used; however, through trial and error one type of glass supplied by a specific glass manufacturer may have been shown to yield the best results. It is suggested that the literature be consulted prior to selecting glass for recording.

Tip Potentials — Detection

During the passage of current the tip potentials of many micropipettes change. Changes in tip potential are indistinguishable from changes in the membrane potential and can therefore represent a serious source of error. To prevent this error the following checks should be made.

(1) While the micropipette is outside the cell, set the offset to zero. In bridge or DCC mode pass a constant current into the bath for about 10 seconds. The current magnitude should be the same as the maximum sustained current likely to be passed during the experiment. When the current is switched off the recorded potential should return to zero within a few milliseconds at most. Some micropipettes either return very slowly to zero potential, or not at all. These micropipettes should be discarded.

(2) Once the experiment is in progress occasionally check the resistance of the micropipette. Changes in tip potential are usually accompanied by changes in micropipette resistance.

Note that the tip potential changes described in this section are happening with a slower time course than the ones described in the *Anti-Alias Filter* section in the **REFERENCE GUIDE: PRINCIPLES OF OPERATION**. The causes of these slow changes in tip potential are unknown.

Tip Potentials — Prevention

Not much can be done to prevent tip potentials from changing but the following may be helpful.

(1) Sometimes the slow changes in tip potentials are worse when standard micropipette holders with an embedded AgCl pellet are used instead of an Ag/AgCl wire. Some holders are all right while other ostensibly identical holders are not. Therefore holders should be tested and selected.

The variability of the tip potentials may in some way be related to pressure developed when the micropipette is pressed into the holder. A narrow hole drilled into the side of the holder to relieve pressure might help. The suction port on the HL-2 series holders provided with the Axoclamp serves this purpose.

(2) Using filling solutions with low pH, or adding small concentrations of polyvalent cations like Th^{4+}, may reduce the size of the tip potential (Purves, 1981) and therefore the magnitude of any changes.

Tip Resistance

Another important aspect of the micropipette is the tip resistance (R_e). This should be as low as possible consistent with good impalements of the cell. Low values of R_e allow for a faster settling time and greater stability of the micropipette.

Settling Time

The decay time constant for the micropipette voltage after a current pulse depends strongly on R_e. Hence, lower R_e values produce faster settling times. As well, high R_e values are sometimes associated with a slow final decay even after C_t has been eliminated.

Stability

R_e of most micropipettes changes with time and with current passing. R_e is affected not only by the magnitude of the current but also by its polarity. In general, micropipettes of lower resistance are more stable during current passing than micropipettes of higher resistance.

Filling Solutions

The best filling solution to use depends on the preparation under investigation and the experience of the investigator. Although KCl gives one of the lowest tip resistances for a given tip diameter it is not necessarily the fastest to settle after a current pulse. K-citrate is sometimes faster.

It is important to be aware that during current-passing large amounts of ions from inside the micropipette can be ionophoresed into the cell. For example, if current is passed by the flow of ion species A from the micropipette into the cell, then after 50 seconds of current at 1 nA (or 1 s of current at 50 nA) the change in concentration of A inside a cell 100 μm in diameter is 1 mM. If A is an impermeant ion, the cell may swell due to the inflow of water to balance the osmotic

pressure. The injection of a permeant ion, such as Cl^-, can significantly alter the equilibrium potential for that ion.

Cell Impalement

Continuously apply current steps and monitor the micropipette resistance. Move the micropipette tip to within several microns of the membrane, and then adjust the capacitance neutralization to give the fastest step response. It is advisable to adjust the capacitance neutralization with the micropipette as close as possible to the final position, since moving the micropipette can change C_{in} and invalidate the setting. It may be wise to slightly under-compensate, otherwise changes in the solution level could lead to oscillations that may destroy the cell. At this point balance the bridge.

Sometimes the cell is impaled as soon as the micropipette is pressed against the cell surface. More often the micropipette is advanced until there is a slight deflection in the tip potential. At this point the cell can be impaled by activating the BUZZ feature or using the CLEAR toggle switch. If these fail, vibrating the micropipette tip by lightly tapping on the micromanipulator sometimes works. When the micropipette penetrates the cell there is a sudden change in the micropipette potential reflecting the intracellular potential. The voltage response to the current steps will be slower and much larger, reflecting the membrane time constant and input resistance.

After impaling the cell, it is often helpful to back-off the micropipette slightly and allow the penetration to stabilize for a few minutes. For some cells you may find it helpful to apply a small DC current to the micropipette (enough to produce several mV hyperpolarization) during the penetration process as this often seems to help stabilize the penetration.

If you are using the two-electrode voltage clamp technique, repeat the same procedure with the current-passing micropipette. There should be little or no change in the response of the voltage-recording micropipette after the current-passing micropipette is in place. If you have trouble gaining access to the cell with the current-passing micropipette, it is sometimes possible to do so by turning on the voltage clamp. Ensure that an appropriate command potential is set and the clamp gain is ≈100 before you try this maneuver.

Note: You may have to consider a potential source of error before impaling the cell with the current-passing micropipette. Clamping very large membrane currents on the order of 10 µA or more can result in resistive coupling between the voltage recording and current-passing micropipettes. This potential problem is discussed further in *TEVC Mode* in the **REFERENCE GUIDE: THEORY OF CLAMP MODES**.

Patch Pipettes

Glass Type and Coating

Pipettes can be obtained from specialty glass houses such as:

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
(415) 883-0128

Noise

The patch pipette may become the dominant source of noise after the other potential contributing noise sources (electronics, pipette holder and membrane seal) have been minimized. 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. 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 Corning #7052 be used for patch pipettes.

Even if one uses electrically superior glass, low noise will not be obtained unless the outer surface of the glass is coated with a hydrophobic substance, such as Dow Corning (Midland, MI) Sylgard #184, dental wax or Sigmacote (a silanizing agent from Sigma Chemical Co., St. Louis, MO). Even though Sylgard is superior, mineral oil may suffice in some situations. All of 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.

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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."

Seal Formation and Whole Cell Recording Configuration

With the patch pipette poised over the preparation, start a rapid repetitive current or voltage pulse. Seals can be obtained either in current or voltage clamp mode. If in current clamp mode, monitor voltage, and in voltage-clamp mode, current. Advance the patch pipette until the resistance increases. This resistance increase occurs when the pipette touches the surface of the cell. Once a cell is encountered, stop advancing the pipette and apply gentle suction. This will often result in the formation of a gigohm seal between the pipette and the cell. There is some variability in the length of time it takes to form this seal. However, the probability of making a successful seal decreases quickly with time.

After the formation of the seal, a pulse of negative pressure will usually break the membrane under the patch pipette resulting in a whole cell recording configuration. Sometimes, it will prove difficult to "break in" to the cell. In this case it may be useful to vary the technique. Another method of breaking into the cell is to use the BUZZ feature on the Axoclamp-2B. When you break into the cell there will be a sudden change in the pipette potential reflecting the intracellular potential. The voltage in response to the current steps will be more rounded and smaller, reflecting the membrane time constant and input resistance.

When using patch pipettes, do not allow the electrode solution to enter the suction port or tube as this will increase the stray capacitance of the patch pipette.

REFERENCE GUIDE: GENERAL INFORMATION

Interfacing a Computer to the Axoclamp-2B

Although the Axoclamp-2B can be controlled manually, the Axoclamp-2B's true potential as a recording instrument is best utilized by interfacing it with a laboratory computer. This section describes the Axoclamp-2B's 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 (EXT. ME1 and ME2 COMMANDS, EXT. VC COMMAND). External commands will be added to internal commands set by the front panel controls.

Logic pulses can be used to trigger BLANK ACTIVATE, CAL. ACTIVATE and STEP ACTIVATE.

Several voltage and current output BNCs are also available (see **REFERENCE GUIDE: INSTRUMENT OPERATION**). Some of these are repeated on the front of the instrument. Thus, 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.

There are several functions of the Axoclamp-2B that can be controlled by computer through the Axoclamp-2B REMOTE connector. These include BUZZ, CLEAR and MODE (see *Remote* in **REFERENCE GUIDE: INSTRUMENT OPERATION**).

Finally, the Axoclamp-2B can be readily integrated into a system containing the CyberAmp.

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 Axoclamp-2B will not be hurt by this load. However, the peak-to-peak swing of signals on the front panel BNCs may be restricted if the equivalent BNC on the rear panel is connected to an interface that is switched off. The same considerations hold true for most tape recorders.

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 power line frequency but also at multiples of it.

The Axoclamp-2B has inherently low hum levels (less than 20 μV peak-to-peak). To take advantage of these low levels great care must be taken when integrating the Axoclamp-2B 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 the ME1 headstage (or to a virtual-ground headstage if used).**

(2) Place the Axoclamp-2B 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 Axoclamp-2B and the radiating equipment can effectively reduce the induced hum.

(3) Initially make only one connection to the Axoclamp-2B: the oscilloscope from the V_1 or 10 V_m outputs. Ground the **ME1** headstage input through a 1 MΩ resistor to the yellow ground connector. After verifying that the hum levels are low, increase the complexity of the connections one lead at a time. Leads should not be draped near transformers 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 Axoclamp-2B via the yellow 0.16" (4 mm) socket on the rear panel. This socket is connected to the Axoclamp-2B's signal ground (*i.e.,* the outer conductors of all the BNC connectors) which is isolated from the chassis and power ground.

(5) If more than one headstage is used, all the headstage cables should run from the Axoclamp-2B 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, earth loops), in practice the ultimate theory is the end result. Following the rules above is the best start. The final hum level can often be kept to less than 100 μV peak-to-peak 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 too prone to accidental alteration.

Power-Supply Glitches

The Axoclamp-2B has been designed to minimize the effects of power-supply transients (glitches). Nevertheless, some glitches do get through. These can cause transients to appear on the voltage and current outputs which may corrupt high-sensitivity recordings (*e.g.,* during fluctuation analysis).

The only completely effective way to gain immunity from power line 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 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.

Model Cells

We recommend that you practice using the Axoclamp-2B with one of the two model cells provided, if you do not need to model your cell exactly. The CLAMP-1U or MCW-1U model cell shipped with your Axoclamp-2B are useful tools for learning the operation of the Axoclamp-2B and subsequently for verifying the correct operation of the Axoclamp-2B and the recording pathway.

The CLAMP-1U Model Cell

The cell and microelectrode components simulate a medium sized cell having an input resistance of 50 MΩ, a membrane time constant of ≈25 ms and microelectrode resistances of 50 MΩ. A switch allows the CLAMP-1U model cell to be configured as (a) BATH mode — two 50 MΩ microelectrodes to ground, or (b) CELL mode — two microelectrodes connected to a 50 MΩ // 470 pF cell. See Figure 10. The case of the model cell is connected to ground. Shielding between the two microelectrode resistors is effected by the body of the switch.

When the switch is in the BATH position, both microelectrode resistors are connected to ground. This is a convenient position for practicing bridge balancing techniques and offset adjustment.

When the switch is in the CELL position, both microelectrode resistors are effectively intracellular. In Bridge or DCC mode you should see exponential voltage responses to steps of current. In dSEVC mode you should be able to clamp the cell at gains of up to 0.8 nA/mV using an HS-2A-x0.1LU headstage, at sampling rates up to 8 kHz. In TEVC mode, use one of the following microelectrode combinations: 1) two x0.1LU headstages, two x1LU headstages, or a x1LU headstage for ME2 and a x0.1LU headstage for ME1. The microelectrode resistances in this model cell are too large for you to practice cSEVC.

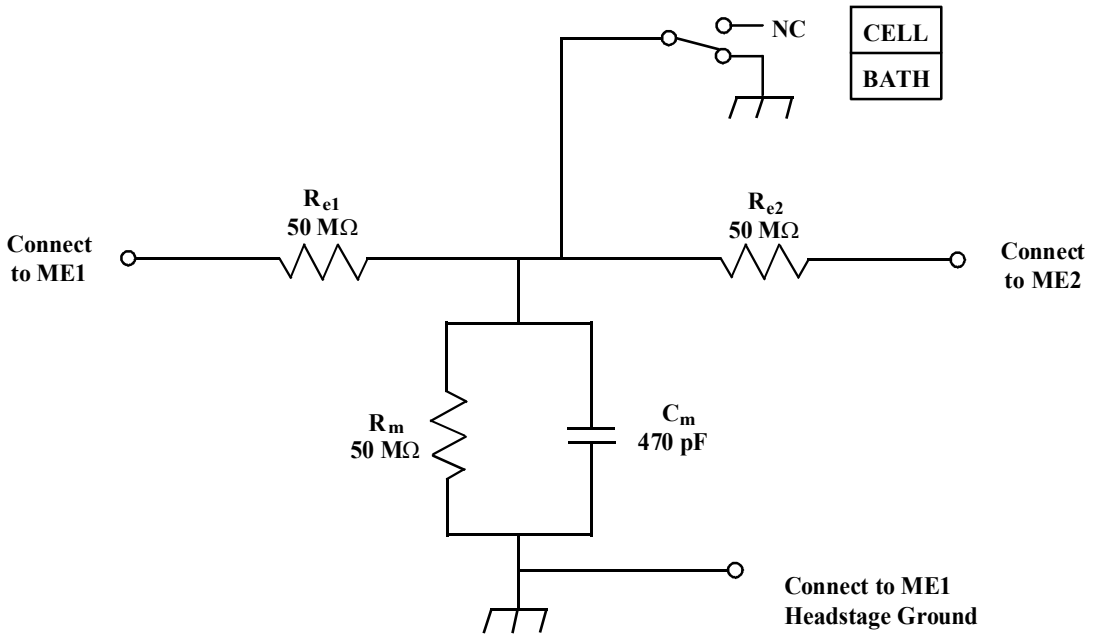

Figure 10. CLAMP-1U model cell

The MCW-1U Model Cell

This MCW-1U model cell simulates a whole-cell recording system (see Figure 11). The membrane time constant is 16.5 ms. The case of the model cell is connected to ground and there is no shielding between the two microelectrode resistors. This model cell is primarily intended to simulate recording from small cells with patch pipettes in cSEVC or dSEVC modes. In this case R_{e2} can be connected to ME2 in order to monitor the true membrane potential.

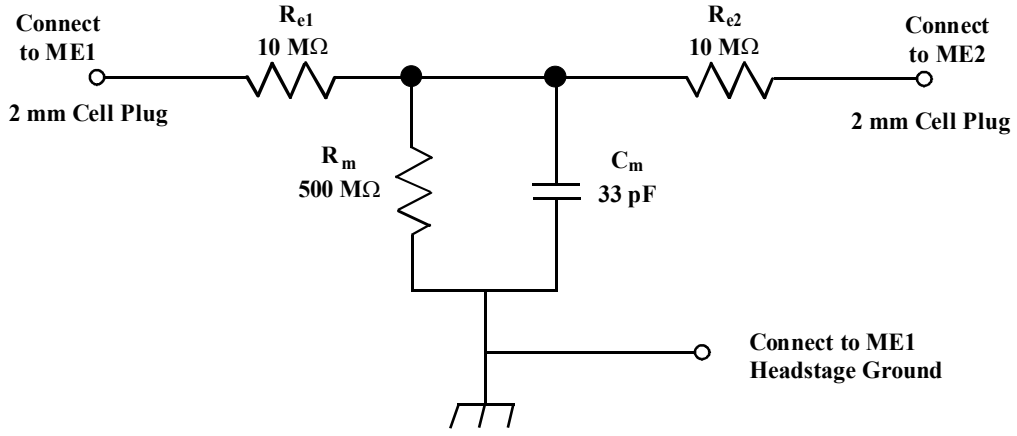

Figure 11. MCW-1U model cell

If the Axoclamp-2B is used in TEVC mode to clamp oocytes, the MCO-1U model cell may be purchased. This model cell mimics the typical characteristics of the oocyte, the recording microelectrodes and the bath electrodes.

Installation of Model Cells

To install the model cell plug it into one or both of your headstages. Connect the gold-plated ground jack to the **yellow** jack on the back of the ME1 headstage using the cable provided. Do not make any connection to the gold-plated jack on the front of the HS-2 or HS-2A headstage — this is connected to the headstage case which in some headstages is driven to the electrode potential.

If you need to model other values, the resistor provided with each headstage can be conveniently used to simulate the microelectrode and the RC cell model can be soldered directly to the free end (see Figure 12). If two-electrode voltage clamping is being practiced it is important to place a grounded shield between the model microelectrodes and between the headstages.

Notes:

1) R_{e1} and R_{e2} are resistors to simulate the microelectrodes.

2) R_m and C_m are a resistor and capacitor to simulate the cell.

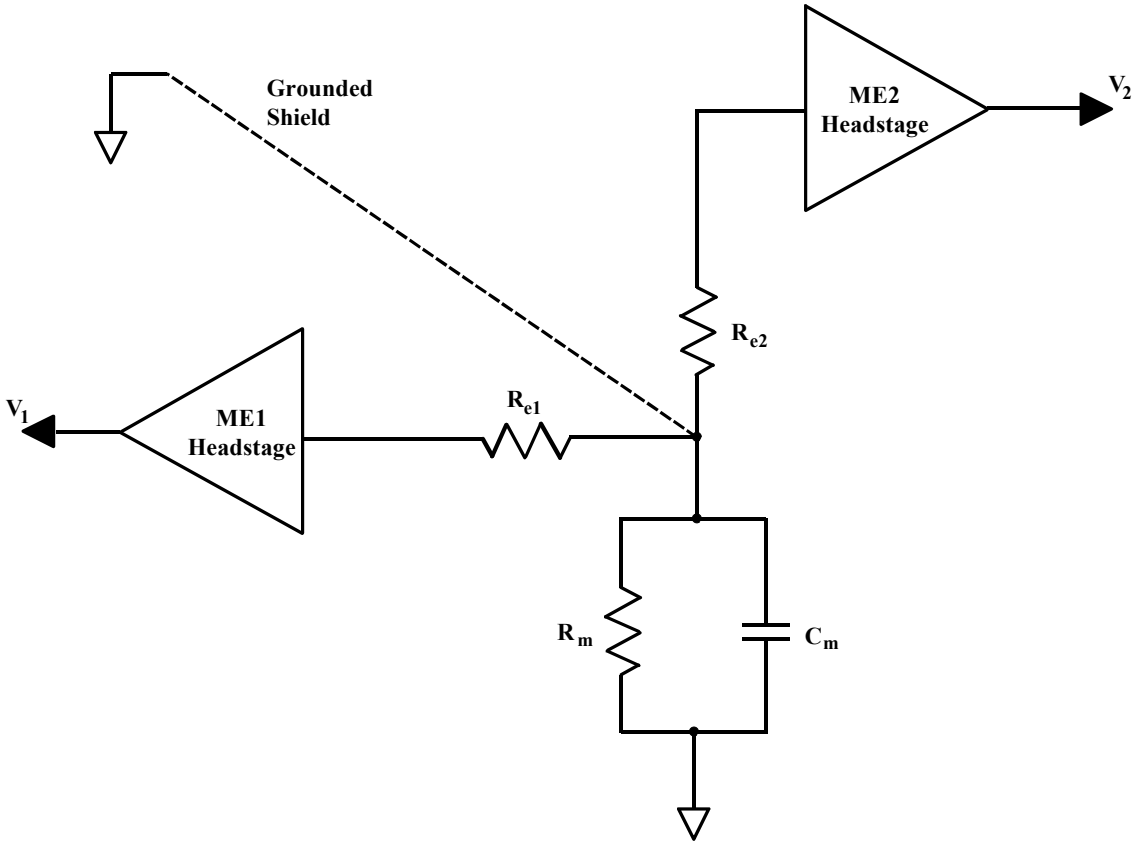

Figure 12. Suggested cell model

Ten-turn Potentiometers

The ten-turn potentiometers used in the Axoclamp-2B are high-quality wirewound types.

An inherent problem of wirewound potentiometers is that the wire elements tend to oxidize. This condition is curable.

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 from the element and restores noise-free operation.

REFERENCE GUIDE: INSTRUMENT OPERATION

Descriptions of the controls and features of the Axoclamp-2B are alphabetically organized in this chapter.

Blanking

A common problem when using stimulating electrodes is that some of the stimulus is directly coupled into the recording microelectrode. This can saturate the coupling capacitors of subsequent AC recording circuits. The saturation effects may take tens or hundreds of milliseconds to subside. The best way to minimize or even eliminate this artifact is at the source, by using small stimuli, isolated stimulators (*e.g.,* Axon Instruments' Isolator-10 or Isolator-11), placing a grounded shield between the stimulating electrodes and the microelectrodes, etc. Often, though, it is not possible to reduce the artifact to manageable levels.

The Axoclamp-2B can circumvent the effects of the stimulus artifact by Blanking. At the moment the logic level of the BLANK ACTIVATE input goes high, the value of V_m is sampled and saved. For the duration of the HIGH signal, this saved value is used instead of the actual potential.

In voltage-clamp modes the voltage-clamp current during the Blanking period will be held at the level which existed at the start of the period. A small deviation from the command potential may develop during the Blanking period as a result of comparing the command to the sampled value of V_m instead of the instantaneous value of V_m. This deviation will only be seen when the Blanking period ends. Usually this deviation is preferable to the situation that can occur if Blanking is not used. If Blanking is not used the artifact picked up by ME1 is treated by the voltage-clamp circuit as an attempt by the cell to change its potential. Therefore, the voltage-clamp circuit causes a current to be passed into the cell to clamp this presumed membrane potential change. If the stimulus artifact is large, the consequent current artifact can be large enough to damage the cell.

The width of the Blanking period should be no longer than the minimum width required to cover the period of the stimulus artifact. It is important not to Blank for longer than necessary since during Blanking no updating of V_m is allowed. Even when Blanking is used, attempts should still be made to minimize the artifact at the source.

Buzz

When BUZZ is activated the amount of capacitance neutralization is increased. If the CAPACITANCE NEUTRALIZATION control is within a few turns of optimum, this extra compensation causes the headstage to go into high-frequency oscillation causing the microelectrode voltage to oscillate.

Depending on the micropipette and the preparation, this method can aid in clearing blocked micropipette tips. When used while the tip of the micropipette is pressing against the membrane, BUZZ may also cause the micropipette to penetrate the cell. The exact mechanism is unknown, but it may involve attraction between the charge at the tip of the micropipette and bound charges on the inside of the membrane.

It is difficult to interpret the operation of Buzz by watching the 10 V_m trace. This is because the x10 gain and low-pass filter on the 10 V_m output strongly affect the amount of headstage oscillation seen. As a quick guide, if the 10 V_m trace is unaffected then Buzz did not succeed, increase the CAPACITANCE NEUTRALIZATION setting until the 10 V_m trace jumps.

The Buzz oscillation can be clearly observed on the V_1 CONT. output.

The duration of the Buzz oscillation is normally equal to the time that the front-panel switch is pressed. Practically, the shortest duration that this switch can be pressed is about 100 ms. For some small cells a long duration Buzz can be deadly. In this case it may be helpful to use an external pulse generator connected to pin 15 of the Remote connector to gate the Buzz oscillation so that it is on for just a few milliseconds. An appropriate duration can be found for most cells that is sufficiently long to allow penetration of the membrane but short enough that the cell is not damaged after penetration.

Alternatively, the hand-held Remote Buzz generator is designed to allow you to conveniently generate Buzz durations between 1 and 50 ms. Plug the Buzz control into the rear-panel REMOTE connector of the Axoclamp-2B. Set the desired Buzz duration on the Duration control of the Remote Buzz unit. Press the button corresponding to the microelectrode you want to buzz. Note that the Duration control is shared by the two microelectrodes. For Buzz durations greater than 50 ms, use the buttons on the front panel of the Axoclamp-2B. Neither the buttons on the front panel of the Axoclamp-2B nor the footswitches use the duration set on the Remote Buzz unit.

To use the FS-3 footswitches, plug them into the 4 mm jacks on the back panel. The red jack labeled "+5 V" is shared by the two footswitches. There is one violet jack for each of the two footswitches.

If a grounded shield adds a lot of capacitance to ME2 the capacitance neutralization range may be insufficient when the L version of either an HS-2A or HS-2 headstage is used and Buzz may not be effective in causing oscillations. In this case it will be necessary to use an M version headstage (see *Headstages* in this chapter).

Calibration Signal

A calibration signal can be simultaneously superimposed on all of the voltage and current outputs (except I_{BATH}.) for the duration of a HIGH signal on the CAL. ACTIVATE input.

For voltage outputs, the magnitude of the CAL. signal is directly equal to the setting of the STEP COMMAND thumbwheel switch. For example, +123.4 will put +123.4 mV on the voltage outputs.

For current outputs, the magnitude of the CAL. signal is 10x the setting of the STEP COMMAND thumbwheel switch. For example, -019.6 will put -196 mV on the current outputs. The equivalent current depends on H. In this example, the CAL. signal of -196 mV would correspond to -19.6 nA for H = x1, -1.96 nA for H = x0.1 etc.

Suggested Use

At the start of a recording sequence, briefly activate CAL. After a short interval, activate the STEP COMMAND. The CAL signal will be a permanent record of the command voltage or current.

Clear

There is one CLEAR switch for each microelectrode. It is used to pass up to ±600 x H nA down the microelectrode. Plus (+) and minus (-) correspond to depolarizing and hyperpolarizing currents, respectively. The CLEAR switch is used for two purposes.

When the micropipette tip resistance goes high this condition can often be cleared by rapidly toggling the CLEAR switch from plus to minus. Because of the large current passed this should only be done extracellularly.

Sometimes micropipette tips press against the cell membrane but fail to penetrate. A quick flick of the CLEAR switch will often force the micropipette to penetrate. Whether to use a hyperpolarizing or depolarizing current depends on the preparation and must be determined by trial and error. Like Buzz, the mechanism for impalement is unknown.

The remote command may be modified to activate the clear currents (See *Remote* in this chapter).

Command Generators

In any mode, level and step commands can be generated internally. Level Commands (one for voltage clamp and one for each microelectrode for a total of 3) are set on precision ten-turn potentiometers. The STEP COMMAND is set on a 3½-digit thumbwheel switch and can be directed to either one of the microelectrodes or to the voltage clamp. An indicator light for each microelectrode illuminates during current commands. External command sources can be used simultaneously with the internal command sources.

Command levels for voltage clamp or current clamp can be obtained from the internal step command generator, from the internal DC command generators, and from external sources.

Step Command Generator

The STEP COMMAND generator can be used either as a current-clamp or voltage-clamp command depending on the position of the DESTINATION switch. If the DESTINATION switch is used to select VC then the magnitude on the thumbwheel switch represents voltage-clamp potential in millivolts irrespective of the headstage current gain (H). If the DESTINATION switch is used to select ME1 or ME2 then the magnitude on the thumbwheel switch represents the number of nanoamperes of current to be injected down ME1 or ME2, respectively. The current range is scaled by the current gain (H) of the headstage. The maximum magnitude on the thumbwheel switch is 199.9. Plus (+) and minus (-) correspond to voltage shifts or currents that are depolarizing and hyperpolarizing, respectively.

The duration for which the STEP COMMAND is activated can be made continuous by switching the EXT./CONT./OFF toggle to CONT. or externally determined by a logic HIGH level on the rear-panel STEP ACTIVATE input. When rotating the thumbwheel switch in continuous mode, be decisive. If the switch is rotated slowly the output will momentarily fall to zero because the switching contacts will pass through an open-circuit state.

DC Command Generators

Separate DC command generators are provided for VC, ME1 and ME2.

The DC command for VC is called HOLDING POSITION. It allows the membrane potential holding position during voltage clamp to be shifted to a value in the range ±200 mV. It is always operative during voltage clamp. Before the voltage clamp mode is selected, the HOLDING POSITION potentiometer is used to set the RMP BALANCE (see the RMP Balance section). The HOLDING POSITION potentiometer is deliberately not calibrated because the exact setting depends on the adequacy of the clamp gain. Instead, the holding position should be read directly from the digital voltmeter displaying V_m. A ten-turn locking dial is used so that once set, the HOLDING POSITION potentiometer can be locked to prevent accidental changes.

The ME1 and ME2 DC commands are called DC CURRENT COMMAND. Each is controlled by a precision ten-turn dial and can be switched by a toggle switch from depolarizing (+) to hyperpolarizing (-) or to the OFF position. An LED illuminates whenever the toggle switch is in the plus or minus position. It also illuminates if the DESTINATION switch is turned to the microelectrode in question and the STEP COMMAND generator is activated either by the EXT./CONT. switch or by a logic HIGH level on the STEP ACTIVATE input. The current is scaled by the current gain (H) of the headstage. If the STEP COMMAND and the DC CURRENT COMMAND are used simultaneously, the total command is their sum.

External Command Inputs

Three external command inputs are provided. These are for setting the voltage-clamp command (EXT. VC COMMAND), the current-clamp command in **ME1** (EXT. ME1 COMMAND), and the current-clamp command in ME2 (EXT. ME2 COMMAND). These inputs are active simultaneously with the internal command generators and do not depend on the position of the DESTINATION switch. The sensitivity of EXT. VC COMMAND is 20 mV/V. The sensitivity of the EXT. ME1/ME2 COMMAND is 10 x H nA/V.

The external command inputs are DC coupled. Therefore, when using the EXT. ME1 and ME2 COMMAND inputs any deviation from zero volts of the external signal source while it is in its OFF state will cause a DC current to flow in the electrode. This can be avoided by using a very high-quality external source which puts out a true zero voltage level in its off state or an isolated external source. If these inputs are driven from the D/A converter of a computer, check the zero offset of the D/A output, and adjust it if necessary.

Mixing Commands

Complex command waveforms can be generated by appropriately mixing the STEP COMMAND, the DC COMMAND and the EXT. COMMAND. For example, the command waveform in Figure 13 can be used to establish the current injected into ME1 by combining the actions of the STEP COMMAND (DESTINATION ME1), the ME1 DC COMMAND and the EXT. ME1 COMMAND input.

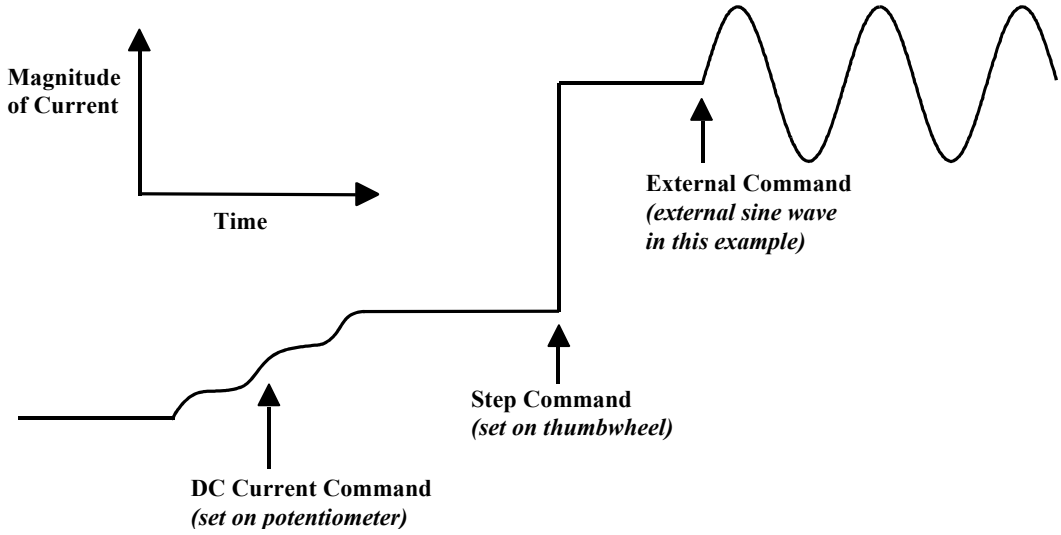

Figure 13. Summation of commands

This figure shows the command potential that would result if all command sources were switched on one at a time and left on.

Headstages

Unity-voltage-gain HS-2 headstages are available in **several current gains.** These cover the range of cell input impedances from less than 1 MΩ to greater than 1 GΩ. Ultrahigh-input impedance versions are also available for ion-sensitive electrodes.

(1) HS-2A and HS-2 Series

HS-2A or HS-2 series headstages are standard. An HS-2A-x0.1LU and an HS-2A-x1LU are supplied with the Axoclamp-2B. Others may be substituted when ordering.

All headstages record voltage at unity gain. For microelectrode #1, ME1, the recorded voltage is available multiplied ten-fold, at the 10 V_m output. For microelectrode, ME2, the recorded voltage is available at unity gain from V_2.

Several headstage current gains (H) are available. Front-panel controls read directly in indicated units when H = x1. All H values are powers of 10. Small H values are used with high-resistance cells and electrodes. Large H values are used to pass large currents.

H = x10, x1, x0.1, x0.01 are for recording and clamping. H = 0.0001 is for ion-sensitive electrodes. The HS-2A headstages are designed to handle larger voltages than the HS-2 headstages. See *Headstages* in **REFERENCE GUIDE: PRINCIPLES OF OPERATION**.

Headstages normally are supplied in the L version (low-noise, low capacitance-neutralization range).

White Teflon collet:	Microelectrode input
Gold Connector:	Shield; case driven or grounded.
Yellow connector:	Signal ground output

The M version can be supplied to compensate larger capacitances.

(2) HS-4 Series

This headstage is an option for the current-passing electrode (ME2) in two-electrode voltage clamp. A VG-2 must be used for current measurement because the internal current-setting resistor is bypassed during two-electrode voltage clamp. Bypassing the resistor allows the output voltage to be applied directly to the electrode.

The HS-4 is supplied in the M version only. When the Axoclamp-2B is not in two-electrode voltage clamp mode, an HS-4 series headstage operates the same as the HS-2 or HS-2Ax1MG series headstages.

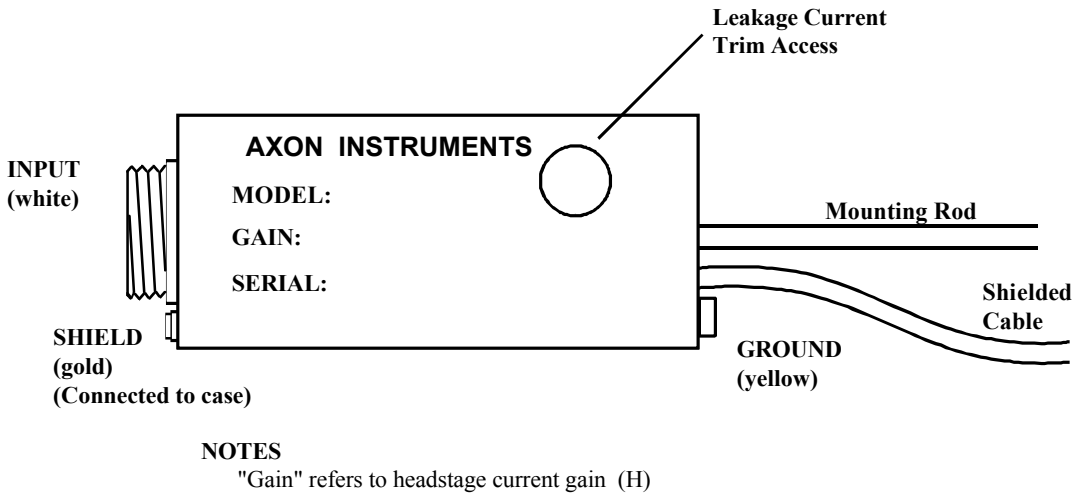

Figure 14. HS-2 and HS-4 headstage connection diagram

(3) VG-2 Series

The virtual ground headstages are optional and are not required for normal operation. Virtual-ground headstages measure the total bath current and are required in two-electrode voltage clamp if the HS-4 headstage is used. They are also useful in clamping the bath when there may be changes in the bath potential caused by changes in temperature or ion concentration in the bath (although the VG-2A-x100 is recommended if this is the only intended use). Virtual Ground output attenuation (VG) specifies the sensitivity. The smaller VG is more sensitive and it is used for low currents.

(4) VG-2A-x100 Series

The VG-2A-x100 bath clamp is optional and may be used to clamp the bath at zero volts. It is used when large current flows in the bath may create significant voltage drops across the resistance of the bath and bath ground.

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.

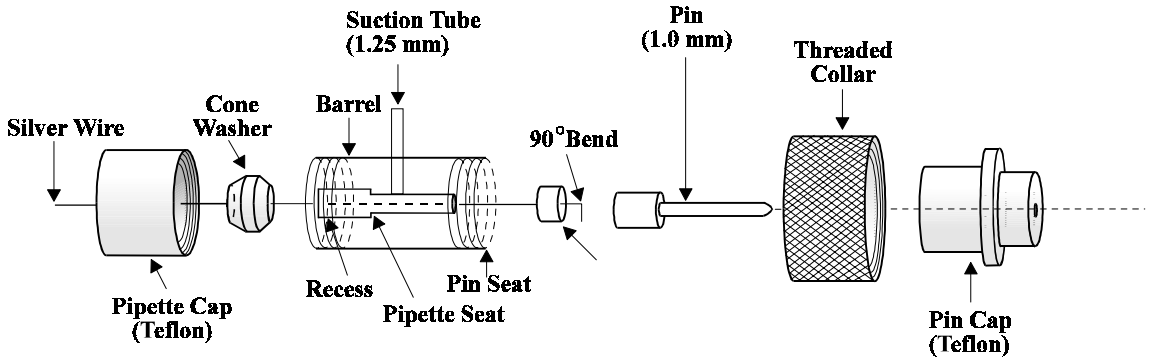

Figure 15. Exploded view of the holder

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.

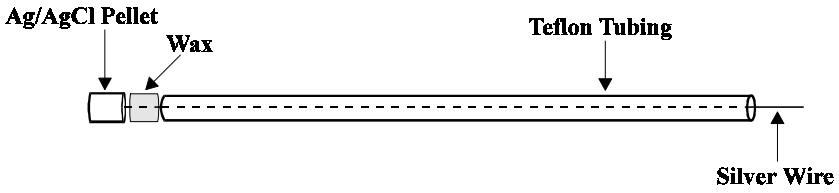

Figure 16. Ag/AgCl pellet assembly

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[1]. 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 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.

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[1]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.

Ionophoresis

When ME2 is not used for intracellular penetrations it can be used for ionophoresis. To set the retaining and pulse currents:

1) Set the desired retaining current on the ME2 DC CURRENT COMMAND control;
2) Switch the DESTINATION switch to ME2. Set the STEP COMMAND equal to the desired pulse current **minus** the retaining current or connect a pulse generator to the EXT. ME2 COMMAND input to set the desired pulse current **minus** the retaining current.

For example, if the retaining current = -5 nA and the ejection current = 40 nA, the ME2 DC CURRENT COMMAND is set to -5 nA and the step command (or EXT. ME2 COMMAND) is set to 45 nA.

A headstage with H equal to x1 is generally useful.

Link-Up

Link-Up enables two Axoclamps running in discontinuous mode to share a common sampling rate. When the Axoclamp-2B is used in dSEVC and DCC modes the voltage across the microelectrode rapidly switches up and down. To an extent which depends on proximity, a second microelectrode used in the same preparation will pick up some switching noise.

If the second microelectrode is used in a continuous mode, the noise picked up can usually be removed by a low-pass filter.

If the second microelectrode is also used in a discontinuous mode (*e.g.,* when two interconnected cells in the same preparation are placed under dSEVC) the pick-up from one to the other can become a problem. The two switching signals mix and a beat frequency signal appears at the difference frequency. When both microelectrodes are switched at similar frequencies the beat frequency signal appears at a low frequency which cannot be filtered out. Worse, in an effort to clamp out the beat signal the clamping circuit passes beat-frequency currents into the cell. There are two ways to avoid this problem.

One method involves placing an extensive grounded shield between the two microelectrodes. The disadvantages are that the shield may be physically difficult to arrange, and it may introduce sufficient capacitance at the headstage inputs to worsen performance.

Another method is to use the Clock Link-Up facility provided with each Axoclamp-2B to synchronize their sampling clocks. A 15-pin connector on the rear panel enables the sampling clock circuits of two Axoclamp-2Bs to be linked by a cable. One Axoclamp-2B becomes the Master and the other the Slave (the relationship is determined by the orientation of the cable).

After Link-Up, whenever both Axoclamps are in DCC or dSEVC modes, the Slave's sampling clock is overridden by the Master's. In all other combinations of operating modes the two Axoclamps remain fully independent. For example, if the Slave is in DCC or dSEVC modes but the Master is in neither, the Slave's sampling clock is re-enabled.

By forcing both Axoclamps to sample synchronously the beat frequency problem is eliminated. At the instant that both Axoclamps sample their microelectrode voltages there will be no pick-up from one microelectrode to the other because the voltages across both must have decayed to near zero in order for the clamps to operate.

Clock Link-Up only affects the sampling clocks. All other functions of the two Axoclamps remain fully independent.

Monitor

When coupled to an oscilloscope, the MONITOR output allows the input to the sampling circuit to be observed. It is essential to observe this signal during DCC and dSEVC to ensure that the microelectrode voltage due to current passing has time to adequately decay at the end of each cycle. An oscilloscope trigger signal at the sample rate is provided for use with the MONITOR signal.

The Monitor signal is derived from V_1 (see Figure 17). After amplification by 10, V_1 is filtered by the Anti-Alias Filter. The output of the Anti-Alias Filter is the input of the sample-and-hold device and the signal provided to the MONITOR output. A baseline correction circuit compensates for shifts in V_1 so that V_{mon} always decays to zero. This prevents V_{mon} from moving off the oscilloscope screen when the holding potential is shifted.

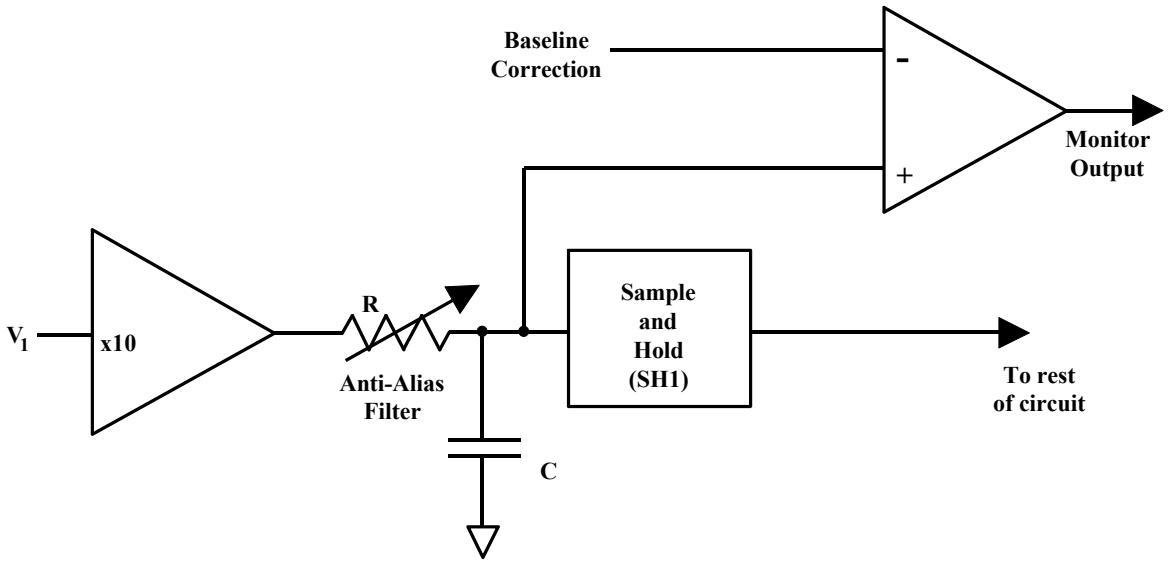


Figure 17. Anti-alias filter and monitor circuit

Output Impedance and Protection

All outputs are protected by 560 Ω output resistors. All outputs can withstand a continuous short circuit to ground or any voltage in the ±15 V range. However, in keeping with normal practice, such short circuits should be avoided.

Panel Meters

Three digital panel meters (DPMs) are provided to continuously display the DC level of some of the important outputs.

(1) **V_m (mV)** indicates the membrane potential in all modes. It is derived from the 10 V_m output. The maximum displayed value is approximately ±600 mV, which is the value which will typically be seen when the **ME1** headstage input is open circuit.

(2) **V_2 (mV)** indicates V_2 in all modes. The maximum displayed value is ±1999 mV. Out-of-range signals are indicated by a partially blanked display, and + or - to indicate polarity.

(3) **I (nA)** can display one of the following currents: I_m, 0.1 x I_2 or I_B (current measured by the virtual ground). The current to be displayed is chosen using the I DISPLAY SELECT switch. Three small switches located under the I DISPLAY are used to change the decimal point to match the headstage current gain so that the display can be read directly in nA for the headstage being used. The H_1 switch is active when the I DISPLAY SELECT switch is in the I_m position; the H_2 switch is active when 0.1 x I_2 is selected; the VG switch is active when I_B is selected. Turn the switches to match the gains of your headstages.

Power Supply Voltage Selection & Fuse Changing

Supply Voltage

The Axoclamp-2B 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 Axoclamp-2B 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.

Remote Control

Selection of the operating mode can be made remotely for computer sequencing of experiments.

Some of the front-panel functions can be activated via the REMOTE connector at the rear of the Axoclamp-2B. These are MODE selection, BUZZ and CLEAR. Possible uses of this facility include using a computer to select the modes, and using hand- or foot-operated switches for BUZZ and CLEAR so that these functions can be used by the experimenter without moving from the microscope.

The selected functions are activated by HIGH logic levels applied to the appropriate pin. New modes are selected and kept after a HIGH level of 1 µs or more. BUZZ and CLEAR are activated for the duration of the HIGH level. Using the REMOTE facility does not disable the front-panel switches.

The pin connections for the Remote connector are as follows:

1.	DIGITAL Ground	9.	Not used
2.	+5 V output	10.	Not used
3.	BRIDGE mode	11.	Not used
4.	DCC mode	12.	CLEAR ME2 "+"
5.	SEVC mode	13.	CLEAR ME2 "-"
6.	TEVC mode	14.	BUZZ ME2
7.	CLEAR ME1 "+"	15.	BUZZ ME1
8.	CLEAR ME1 "-"		

To use the Remote controls, the external control signals can be wired to a 15-pin D-type connector which can then be plugged into the Remote connector on the rear panel.

+5 V is provided for wiring up any remote switches you may use. **Do not short circuit this supply.** The Mode Select inputs (pins 3-6) have 50 kΩ input resistances; the other inputs (pins 7, 8, 12-15) have 7 kΩ input resistances.

The FS-3 footswitches provided with the Axoclamp-2B consist of a pair of normally open switches for activating BUZZ of each microelectrode. If footswitches are not convenient you can easily connect your preferred switches by following the wiring diagram in Figure 18.

For remote operation of microelectrode 1 BUZZ and microelectrode 2 BUZZ.

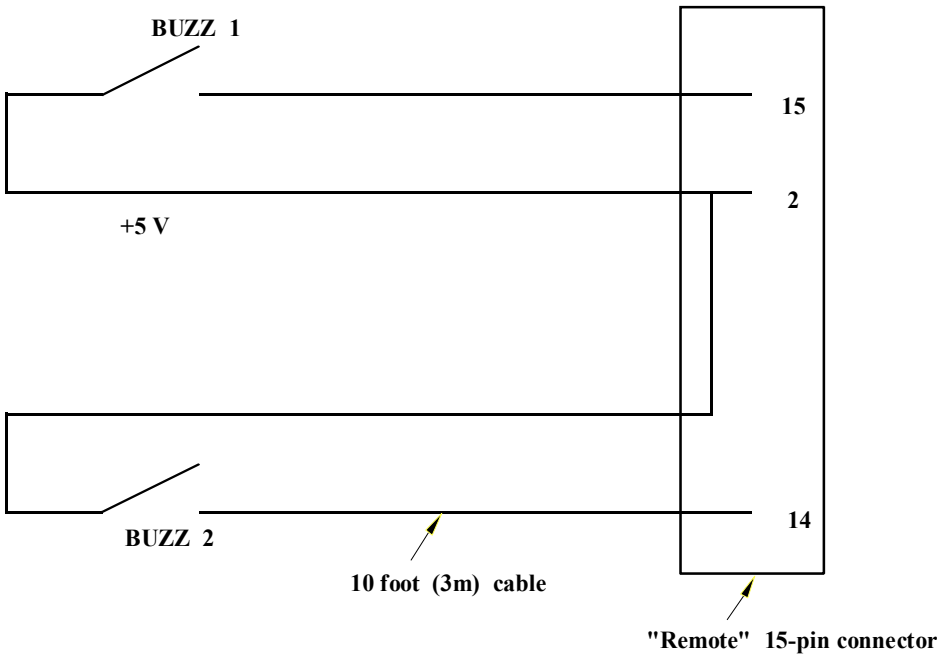


Figure 18. External switch wiring diagram

If you want to use some of the pins on the rear-panel remote connector to remotely select the operating mode or activate the CLEAR currents, you will have to remove the cover from the connector on the Remote Buzz unit and solder your inputs to the appropriate spare pins on this plug.

RMP Balance

The (Resting Membrane Potential) RMP Balance indicator enables you to preset the voltage clamp command while you are recording the resting membrane potential in the current-clamp mode. When you switch into voltage-clamp mode the cell membrane will automatically be clamped at the cell's resting membrane potential, irrespective of the clamp gain.

The two indicator lights for monitoring resting membrane potential are used in two ways.

Before switching into a voltage-clamp mode the HOLDING POSITION potentiometer is adjusted until the two LEDs are equally dim (nulled). At the null point, the HOLDING POSITION equals the currently recorded membrane potential. This ensures that when a voltage-clamp mode is selected the membrane potential will be held within a few millivolts of RMP in the absence of voltage-clamp commands from other sources. When adjusting the HOLDING POSITION control before voltage clamping, the sensitivity of the null point is affected by the voltage clamp gain.

During voltage clamp the RMP BALANCE lights provide a quick indication of when the cell is being held at its resting level. That is, the RMP Balance lights are nulled at this point.

Triggered Clamping

In some experiments it is desirable to switch into voltage clamp only when a specific event threshold is reached. For example, it may be desirable to switch into voltage clamp when the unclamped action potential goes above a predetermined level.

To do this an external device must be used to detect the event and signal its occurrence by putting out a logic HIGH. The logic HIGH is then applied to pin 5 or 6 of the REMOTE connector on the rear panel of the Axoclamp-2B. The Axoclamp-2B will then remain in voltage-clamp mode until the logic HIGH is removed from pin 5 or 6 and a separate logic HIGH applied to pin 3 or 4 of the REMOTE connector.

REFERENCE GUIDE: PRINCIPLES OF OPERATION

Anti-Alias Filter

A property of all digital sampling systems is that noise in the input signal at frequencies greater than 0.5 of the sample rate (f_s) is folded down to appear as extra noise in the bandwidth from zero to 0.5 of f_s (see *Noise in DCC and dSEVC Modes* p. 93). This phenomenon is known as aliasing.

Aliasing can be overcome by filtering the input signal before sampling, thereby reducing the high-frequency noise content. However, this filtering procedure degrades the dynamic response of the input signal and when used with an ideal microelectrode worsens the clamp performance.

The voltage across a real microelectrode often has a two-phase decay after the end of a current pulse, either because of redistribution of ions in the tip, or because of the distributed nature of the capacitance through the wall of the microelectrode (see Figure 9). The final stages of the decay may often be so slow that additional delay introduced by a filter used to prevent aliasing (an Anti-Alias Filter) causes insignificant worsening of the dynamic response.

The ANTI-ALIAS FILTER of the Axoclamp-2B is a first-order low-pass filter that reduces the noise on the headstage input. The ANTI-ALIAS FILTER can be used by the experimenter to trade off the noise recorded in DCC and dSEVC modes against the dynamic response. That is, increasing the ANTI-ALIAS FILTER setting decreases the noise but can lead to instability in dSEVC and can make it more difficult in DCC to balance the response to a current step.

The ANTI-ALIAS FILTER also has an effect in the continuous modes. It acts as a low-pass filter on the voltage recorded by ME1 and sometimes can be used to advantage. The effects during TEVC and cSEVC are the same as those due to a slow voltage-recording microelectrode.

Rotating the ANTI-ALIAS FILTER control clockwise logarithmically increases the amount of filtering. In the fully counterclockwise position the filter time constant is 0.2 µs and the discontinuous clamp responses are unaffected. In the fully clockwise position the filter time constant is 100 µs (16 kHz bandwidth). There is a maximal reduction in noise but the maximum sampling rate which can be achieved is severely limited (to about 1 kHz or less).

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 the system ground (on the assumption that the bath is also at ground). This assumption may not be true if Cl^- concentration or temperature of the bathing solution is significantly changed, there is restricted access from the extracellular space to the grounding point, or 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 (Rb) could be as much as 10 kΩ, although with care it is not difficult to achieve Rb values less than 1 kΩ.

In a simple TEVC setup, the voltage drop across Rb is indistinguishable from the membrane potential. That is, the potential recorded by the voltage-recording micropipette (V1) is the sum of the transmembrane potential (Vm) and the bath potential (Vb). Problems arise if the product of the clamp current (I2) and Rb is significant. For example, for I2 = 5 μA and Rb = 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 and other responses would be seriously affected.

To faithfully record Vm, either Vb must be made equal to or nearly equal to zero, or the value of Vb must be independently measured and subtracted from the potential recorded by ME1. In some rare circumstances it might be necessary to use the more complicated procedure of series resistance compensation.

The following four 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.

(1) Minimize R_b

Steps should always be taken to minimize R_b. 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 Ringer's solution (such as an oocyte) are on the order of 150-200 Ω. This is a given, and 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's solution 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.* 3 M 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 Bridge mode by placing both micropipettes in the bath. Put the micropipette connected to ME1 on the far side of the micropipette connected to ME2, away from the bath electrode. A 100 Hz, 10 V_{p-p} square wave command delivered to the EXT. ME2 COMMAND input produces a 1 μA_{p-p} current. The response measured with the micropipette connected to ME1 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.

(2) Clamp V_b Using a Bath Clamp or Virtual Ground

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

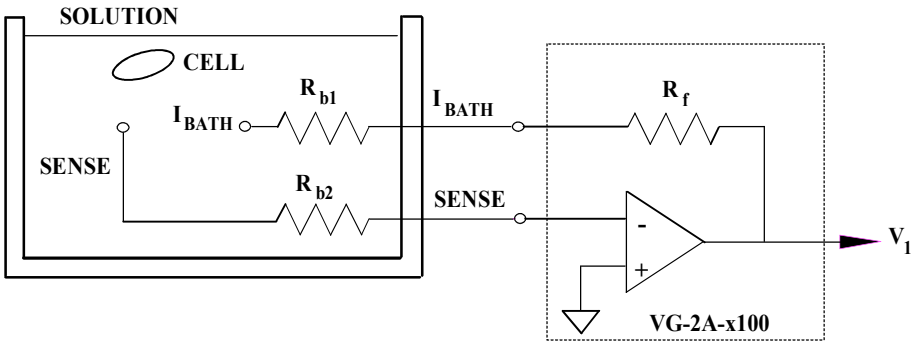


Figure 19. Bath clamp

One electrode (SENSE) is a voltage-sensing electrode. It is placed in the 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 also placed in the 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}.

This configuration is particularly recommended for voltage clamping oocytes. The VG-2A-x100 bath clamp is required for the fastest responses and guaranteed stability. Connect this headstage to the BATH-CLAMP PROBE (I) input.

(3) Independently Measure V_b and Subtract it from the ME1 Potential

When an HS-2A headstage is connected to the rear-panel BATH PROBE (V) connector, measurements by both ME1 and ME2 are automatically made relative to the potential recorded by this headstage. Therefore V_m is unaffected by any changes in V_b produced by line-frequency pickup or by changes in

the temperature or ionic composition of the bath solution. If V_b is not measured the system automatically reverts to using the system ground as the reference potential.

Any of the HS-2A or HS-2 series headstages can be used to record V_b. Headstage gains of x1, x0.1 and x0.01 are suitable. The x10 headstage can also be used, although at the risk of slightly greater high frequency noise.

A broken 3 M KCl-filled microelectrode or a blunt patch pipette filled with the normal external solution works well as an extracellular bath microelectrode. During recording it is positioned close to the cell, and is shielded from the current-passing electrode. The bath microelectrode cannot be used for current passing.

Grounding

Irrespective of whether or not the bath potential is measured, the preparation bath should be grounded by directly connecting it to the yellow ground connector on the back of the ME1 headstage (or to a virtual-ground headstage if used).

Bandwidth of V_b Measurement

The V_b bandwidth is internally filtered a -3 dB cutoff of 300 Hz before it is fed back and subtracted from the measurements made by ME1 and ME2. The full-bandwidth voltage recorded by the bath microelectrode is available at the V_{BATH} OUT connector.

To be effective in compensating for R_s errors the frequency response of a bath voltage electrode should match the voltage recording electrode frequency response. At the same time it is most important for the bath voltage signal to be appropriately attenuated at high frequencies, since this signal is not inverted and, thus, presents a positive feedback pathway that can potentially destabilize the clamp. For optimum voltage-clamp performance, the bandwidth of the bath potential is limited to 300 Hz before it is subtracted from the potentials recorded by ME1 and ME2 (see Finkel & Gage, 1985). Therefore V_b will be effective for series resistance compensation only up to frequencies of 300 Hz. This may not be sufficient for some purposes. In this case actively clamping the bath potential is a superior alternative method for R_b compensation.

(4) Compensate the Series Resistance

It should be emphasized again that there is no substitute for reducing R_b at its source. Failing that, actively clamping the bath potential using a virtual-ground headstage is a simple, low noise and non-destabilizing method for reducing the *effect* of R_b. The combination of these two efforts should substantially eliminate the errors attributable to current flow across R_b. Nevertheless, other contributors to resistance in series with the membrane (R_s) might remain internal to the cell and in the membrane itself. The technique of series resistance compensation can be used to minimize the effect of this remaining component of R_s. However, in most experiments this remaining component of R_s is small and can be safely ignored. If this is not the case, series resistance compensation can be used. The Axoclamp-2B allows for the implementation of this technique, but full support requires that you provide an external potentiometer circuit.

How can you know that the responses recorded from your cells are affected by a cellular component of R_s? In an isopotential cell, the time-course of the membrane current (I_m) transient should be the same as the recorded membrane potential transient (V_m). If R_s is significant, then V_m will be faster since it will include the voltage drop ($I_m R_s$) across R_s. (Internal to the cell, but impossible to record,

the true time-course of the membrane potential will follow the time-course of the I_m transient.) Ideally, R_s can be completely compensated for by adding the voltage, $I_m R_s$, to the clamp command potential.

However, the difference in time course between the V_m and I_m transients is not always an indication that there is a membrane component of R_s. In a non-isopotential cell, for example a neuron with an axon and dendrites, the true membrane potential will in fact settle faster in response to a step voltage command than will the membrane current. In this situation, the presence of a series resistance will exaggerate the difference in time courses. This will make the series resistance compensation technique described below extremely difficult to implement, if not impossible.

To implement compensation, you must connect a 10 kΩ external potentiometer between the I_2 (or I_{BATH}) output and ground. The wiper must be connected back to the R_s COMP. input on the rear panel. To reduce the sensitivity of this circuit, a resistor can be placed in series with the potentiometer.

It is difficult to measure R_s. The simplest way to optimize the setting of the external compensation potentiometer is to apply a repetitive step command in TEVC mode while observing the current transient. Slowly advance the potentiometer setting until the current transient is as fast as possible, without overshoot. The capacitance neutralization controls and the voltage-clamp controls might need to be iteratively adjusted during this procedure.

Since R_s compensation represents positive feedback it can potentially destabilize the clamp and will increase the current noise. Both these problems can be alleviated by filtering the feedback signal (a single-pole filter is usually adequate). The filter cut-off frequency should be the same, or less than, the bandwidth of the ME1 electrode. To avoid unexpected oscillations, be sure to set the potentiometer to its minimum before turning on the voltage clamp.

Note that unlike the R_s compensation described here for TEVC mode, R_s compensation in cSEVC mode is fully supported by the Axoclamp-2B (see page 83). This is because the main contributor to R_s in a cSEVC experiment, the microelectrode, is always present and usually needs to be compensated.

Summary

In summary, we recommend that in experiments where the voltage-clamp current is substantial you should take steps to minimize the value of R_b by appropriately selecting the bath electrodes. If this is insufficient, actively clamp the bath potential (V_b) using a bath-clamp or virtual-ground headstage. If for some reason this approach is inappropriate, consider using an HS-2 series headstage to measure and subtract V_b from the recording pathway. Finally, in the rare situation where there is a membrane component of R_s sufficient to affect the recordings, compensate for this effect by providing and setting an external potentiometer circuit.

Capacitance Neutralization and Input Capacitance

The high-frequency performance of ME1 and ultimately the voltage clamp is reduced by the presence of stray capacitance at the headstage amplifier input (C_{in}). 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 microelectrode resistance (R_e) acts as a low-pass filter for signals recorded at the tip of the microelectrode. For optimal clamp performance at high frequencies this RC time constant must be made as small as possible. Two techniques may be used to increase the recording bandwidth.

The first and simplest step is to use voltage-recording microelectrodes with the lowest possible resistance compatible with stable recording and to take steps to minimize the contribution to C_{in} by the capacitance of the microelectrode (see the **MICROELECTRODES** chapter). The second step is to minimize and then if necessary neutralize C_{in}.

Primary Method for Neutralizing C_{in}

A special technique is used in the headstages to keep the contribution to C_{in} from the input amplifier as small as possible. The technique is known as "bootstrapping." Unity gain feedback is used to reduce the component of stray capacitance that exists between the amplifier input and its power supplies and case. Sophisticated circuitry is used to superimpose the unity-gain output of the buffer amplifier back onto its own power supplies and the headstage case, fixing 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 eliminates the high-frequency current loss through the power supply capacitance, thereby increasing the bandwidth. Since the power supply capacitance is present whether or not the power supply is bootstrapped, there is no noise penalty due to implementing this technique.

Secondary Method for Neutralizing C_{in}

In some cases the steps discussed above may not be sufficient to decrease the RC time constant of the voltage-recording microelectrode, particularly in situations where high resistance microelectrodes must be used. For this reason an effective, though less desirable, technique is provided that can electrically reduce the *effective* magnitude of C_{in2}. The technique is known as "capacitance compensation", "negative capacitance" or "capacitance neutralization." A compensation amplifier at the output of the unity gain buffer drives a current injection capacitor connected to the input. At the ideal setting of the compensation-amplifier gain, the current injected by the injection capacitor is exactly equal to the current that passes through C_{in2} to ground.

To use the capacitance neutralization circuit the voltage response to a current step must be observed. Advance the capacitance neutralization control as far as is 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 microelectrode.

Use of capacitance neutralization is less desirable than physically minimizing C_{in2}, since the neutralizing circuit adds noise to the voltage signal. However, Axon Instruments has ensured that the added noise is minimal by using low-noise amplifiers and small injection capacitors. The low-noise "L" series headstages use a 3 pF injection capacitor. The medium-noise "M" series headstages use a 10 pF injection capacitor. The M series headstages are typically only used for output (current-passing) in a TEVC setup.

It is important to recognize that the capacitance neutralization circuit is not more than 90% effective even for ideal microelectrodes. This is because of the finite frequency responses of the headstage amplifiers and the capacitance neutralization circuit, and also because C_{in2} does not behave ideally as a linear lumped capacitor. Consequently, the amount of C_{in2} 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 microelectrode to the input. If possible, plug the microelectrode holder directly into the input. Use shallow bathing solutions. Avoid having grounded objects near the microelectrode. Do not ground the headstage case.**

Grounding and Driven Shield

If metal objects (such as the microscope) must be very near the microelectrode, they may be disconnected from ground and connected to the gold shield socket in the headstage (see *Headstages* in this chapter). This technique can improve the microelectrode response speed. However, in DCC and *dSEVC* modes there may be an increase in the amount of switching noise picked up by independent recording microelectrodes, if used.

It has been common to reduce the effective C_{in2} by placing a driven shield around the voltage recording microelectrode and extending close to the tip of the microelectrode. This procedure is not recommended as it greatly increases the voltage noise level which under voltage clamp is manifest as increased current noise.

Current Measurement

The current injected down each microelectrode is independently measured. The measurement is true. Thus, if the microelectrode blocks, the measured current falls to zero even though a current command may exist.

Two current outputs apply to ME1. I_m is the membrane current while I_1 CONT. is the instantaneous current in ME1. In continuous modes (Bridge, cSEVC and even TEVC) I_m and I_1 CONT. are identical. However, in discontinuous modes (*i.e.,* DCC and dSEVC) I_m and I_1 CONT. are different. I_1 CONT. switches from zero to some finite value at the sample rate. This is because for 30% of each period ME1 is used for passing current while for the remaining 70% of each period no current is passed and the IR voltage drop due to the previous current is allowed to passively decay (see DCC and cSEVC sections). On the other hand, I_m is the true membrane current. It is recovered from the instantaneous current by a circuit which samples the current pulses, retains the samples during the passive-decay period, then scales the samples to yield the average current for the whole period. The I_m output is smoothed by the output filter.

The current in ME2 is labeled I_2 and 0.1 x I_2. The attenuated version is useful when large currents are present during TEVC mode. The unattenuated version has lower noise.

The gains of the current measurement circuits depend on the headstage current gains (H). It is (10 ÷ H) mV/nA for I_m and I_2, and (1 ÷ H) mV/nA for 0.1 x I_2.

The whole current into the bath can be separately measured using a virtual-ground headstage (see *Virtual Ground Current Measurement* p. 97).

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 the signal 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 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.

Using cSEVC in a Macropatch Configuration

The 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" or "inside out." The patch-clamp pipette current is positive if it flows from the headstage through the tip of the pipette into the patch membrane.

Cell-Attached Macropatch

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 patch 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 patch pipette current flows through the patch pipette, across the patch membrane into the cell. Therefore a *positive* current is *inward*.

Inside-Out Macropatch

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 patch 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 patch 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*.

Summary

1) *Positive* current corresponds to:

Cell-attached macropatch	patch inward current
Inside-out macropatch	patch inward 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 macropatch	hyperpolarizing
Inside-out macropatch	hyperpolarizing
Whole-cell voltage clamp	depolarizing

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

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

Filtering with External Filters

In cSEVC and TEVC modes, an external fourth-order Bessel filter is advisable, because the current noise spectrum rises rapidly with frequency. The CyberAmp 320 or CyberAmp 380 from Axon Instruments can be used to provide this filtering.

Headstages

Compatibility of the HS-2 and HS-2A With the Axoclamp-2B

In many cases an HS-2 headstage may be substituted for an HS-2A. However, in TEVC mode, an HS-2 headstage in the ME2 position might cause the ME2 output to latch up (*i.e.,* the output voltage might become stuck at plus or minus 130 V). This will not occur with an HS-2A headstage.

The performance of ME1 in either current clamp or single-electrode voltage clamp mode is the same for HS-2 and HS-2A series headstages.

General Characteristics

The HS-2A and HS-2 unity gain headstages buffer the high impedance of the microelectrode, making the potential recorded by the microelectrode available to the rest of the circuitry. It also provides the means for injecting current into the microelectrode and for neutralizing the input capacitance.

Current in each microelectrode is continuously measured during both voltage clamp and current clamp. This measurement does not include currents from sources other than the microelectrode (*e.g.,* hum, ionophoresis, the other microelectrode) and indicates zero if the microelectrode blocks.

The Meaning of H

A precision resistor (R_o) inside the headstage sets the headstage current gain (H). The particular value of H used affects the Bridge range, the sensitivity to current commands, the sensitivity of the current monitors and the gain in SEVC mode. The effects are clearly marked on the front and rear panels, and since they always appear in multiples of ten, they are easy to calculate. For your convenience, Table 1 summarizes these effects. Note that voltage commands during voltage clamp and recorded voltages are not affected by the headstage current gain value.

TABLE 1
How H affects control and measurement ranges

H(1)	x10	x1	x0.1
R_o	1 MΩ	10 MΩ	100 MΩ
Max. Bridge Balance	10 MΩ	100 MΩ	1000 MΩ
Max. Step Command	±1999 nA	±199.9 nA	±19.99 nA
Max. DC Current Command	±1000 nA	±100 nA	±10 nA
Ext. Command	100 nA/V	10 nA/V	1 nA/V
Max Total Current(2)	6000 nA	600 nA	60 nA
I Output	1 mV/nA	10 mV/nA	100 mV/nA
Max. Gain in dSEVC	1000 nA/mV	100 nA/mV	10 nA/mV
Max. Gain in cSEVC	10000 nA/mV	1000 nA/mV	100 nA/mV
Max. Gain in TEVC	10000 V/V	10000 V/V	10000 V/V

(1) For H = x0.01 replace MΩ by GΩ, nA by pA in x10 column
For H = x0.0001 replace MΩ by GΩ, nA by pA in x0.1 column
For H = x100 replace MΩ by kΩ, nA by µA in x0.1 column

(2) Measured with electrode resistance $R_e = R_o$

Guidelines for Selecting a Headstage

The H value required depends on the typical input resistances, R_{in}, of your cells. The recommended values given in Table 2 are for micropipettes. When patch pipettes are used, H = 0.1 should be selected as a matter of course.

TABLE 2
Recommended H values for various cell input resistances

Some overlap in these recommendations is allowable. The guiding principles are these:

H	=	x10	for	300 kΩ	<	R_{in} <	3 MΩ
H	=	x1	for	3 MΩ	<	R_{in} <	30 MΩ
H	=	x0.1	for	30 MΩ	<	R_{in} <	300 MΩ
H	=	x0.01	for			R_{in} >	300 MΩ
H	=	x0.0001	for	ion-sensitive electrodes			

(1) For maximum sampling rates in dSEVC and DCC modes use the **largest** feasible H value. (This is because the current-passing response is best with **low** values of R_o.)

(2) A limitation on using large H values is that as R_o becomes smaller the input leakage current of the headstage becomes more prone to increase with time and temperature (see *Input Leakage Current* discussion later in this section).

(3) A further limitation on using large H values is that if R_o is less than the microelectrode resistance, R_e, the high-frequency noise is worse.

(4) The H value sets the current-passing sensitivity in Bridge and DCC modes and the Gain in SEVC modes. Hence it should be chosen for sensitivities suitable for your cell. These sensitivities are listed in Table 1 above.

(5) If $R_e >> R_{in}$ a smaller H value should be favored.

Capacitance Neutralization Range

Headstages are available with L or M suffixes representing low and medium ranges respectively of Capacitance Neutralization (see Table 3). The increased Capacitance Neutralization range is a trade-off against microelectrode noise. The L version of the HS-2A or HS-2 has the lowest noise and performs close to the theoretically predicted thermal noise of the electrode. The HS-2M has about 25% more noise. The M series headstages are occasionally used when tracking microelectrodes through long lengths of tissue, *e.g.,* when recording from an intact brain. In a two-electrode voltage clamp, noise in the ME2 microelectrode is less important than noise in the input (voltage recording) microelectrode, and it is sometimes useful to have a larger compensation range because in a two-electrode voltage clamp it is common to place a grounded metal shield near the current-passing microelectrode to prevent coupling of its signal into the voltage recording microelectrode.

TABLE 3

	L	**M**
CAP NEUT RANGE:		
in ME1 Slot	-1 to 7 pF	-2 to 20 pF
in ME2 Slot	-1 to 14 pF	-2 to 40 pF

General Rule for Choosing an HS-2(A) Headstage

The general rule for intracellular microelectrode recording is that the resistor in the headstage (R_o) should be between 1/3 and 3 times the value of the electrode resistance, R_e. If the R_o value is less than 1/3 R_e, the wideband noise will increase and the headstage leakage current might cause DC errors. If the R_o value is greater than 3 times R_e, the voltage drop across R_o during current passing might be excessive and the bandwidth might be compromised.

If the value of R_o is higher or lower than the recommended range, there is a gradual deterioration in performance. There is no catastrophic diminishment of performance, and in many instances, the user will be blessed with adequate performance even if R_o is as small as 1/10 or as large as x10 R_e.

USE	**RECOMMENDATION**
ION-SELECTIVE ELECTRODES	x0.0001 for the recording electrode and x0.1 for the reference electrode.
NORMAL INTRACELLULAR (BRIDGE) RECORDING	Follow the general rule.
EXTRACELLULAR RECORDING	Since there is no current passing, there is no upper limit on R_o. For example, the x1 and x0.1 headstages are equally suitable for a 1 MΩ extracellular electrode.
dSEVC and DCC	x1 and x0.1 give the best dynamic performance. x0.01 will not cycle as fast.
cSEVC	x0.1 is usual.
TEVC	Usually use the largest reasonable H value for the current-passing microelectrode (ME2) and the lowest reasonable H value for the voltage-recording microelectrode (ME1). For example, use x10 or x1 for ME2 and x1 or x0.1 for ME1. Use the "G" version for ME2 if practical. The G version has a grounded case, thus minimizing the crosstalk between the two electrodes. The only reason not to recommend the G version is if it is intended to use the headstage on occasion as the voltage-recording (ME1) headstage. When used this way, the larger input capacitance of the G version contributes to increased wideband input noise.
IONOPHORESIS	To electrically eject charged molecules out the microelectrode tip into the preparation, it is best to use low resistance microelectrodes and high H values. x1 is the best general-purpose headstage for ionophoresis of drugs or dyes.

OOCYTE CLAMP	This is a TEVC application using very large cells. The cell input impedance could be as low as 100 kΩ in parallel with several hundred nanofarads. A popular combination is the HS-2A-x1LU for ME1, the HS-2A-x10MGU for ME2 and the VG-2A-x100 for clamping the bath potential. The VG-2A is not required, but it is advisable if the total membrane current is 5 µA or greater. An HS-4 series headstage for ME2 can be used. This allows slightly more voltage to be applied to the electrode but in practice there is little or no observable benefit.

HS-4-x1MGU Headstage

In TEVC mode the current-measuring resistor R_o is bypassed in this headstage. If the HS-4 is used in any clamp mode other than TEVC, the current-measuring resistor is switched into the circuit. Consequently, the HS-4 performs like an HS-2A-x1MGU headstage.

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 headstage 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 Axoclamp-2B main unit during TEVC is ±130 V. This compliance is usually sufficient to drive the current through the current-passing microelectrode 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 series 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

Two of the three sockets in the headstage are Teflon-insulated, as shown in Figure 12. These are standard-diameter sockets.

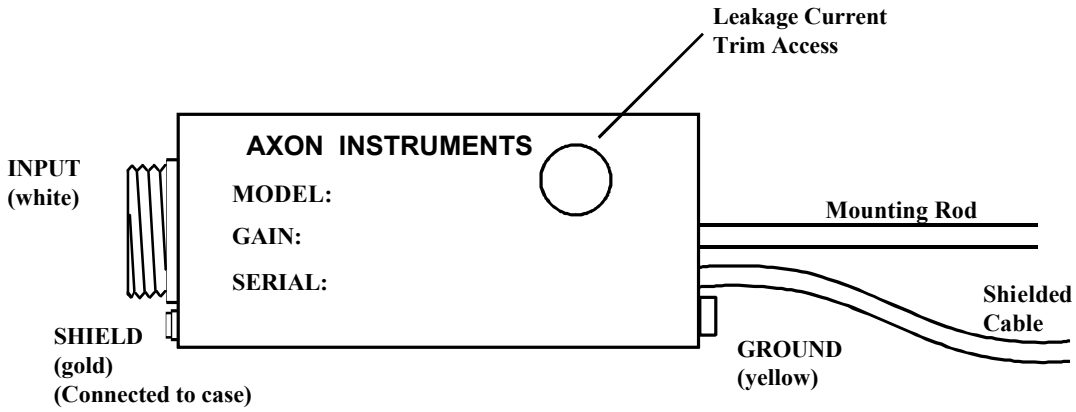
Microelectrode Input Connector (White)

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 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.



NOTES

"Gain" refers to headstage current gain (H)

Shield Drive Connector (Gold)

The Shield drive is connected to the gold-plated guard socket and to the case of the x1L, x0.1L, x0.01M and x0.0001M headstages. 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 (see notes in *Microelectrodes for Fast Settling* section). 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 impedance 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 x1MG, x10MG 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.

Interchangeability

Any unity-gain headstage in the HS-2 or HS-2A series can be used for ME1 or ME2. The equipment will not be damaged if headstages are exchanged while the Axoclamp-2B is switched on.

Cleaning

To clean salt spills from the input connectors wipe with a damp cloth. Avoid spilling liquids on the headstage.

Input Leakage Current

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 H value. As a result, an undesirable DC leakage current is injected into the microelectrode.

Careful consideration of this problem has been applied throughout the design of the Axoclamp-2B and the overall DC offset has been made as insensitive as possible to the drift in the integrated circuits. As well, special low-drift integrated circuits have been used in all critical positions. The magnitude of the DC leakage current increases with H. This normally introduces no greater error in the DC offset voltage developed across the microelectrode or the cell membrane because larger H's are usually used with lower-resistance cells and microelectrodes.

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

100 pA	for	H = x10
10 pA	for	H = x1
1 pA	for	H = x0.1
1 pA	for	H = x0.01
10 fA	for	H = x0.0001

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 (see Table 2). (The shift in V_m is calculated from input current x R_{in}.)

How To Trim the Input Leakage Current To Zero

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 physically 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 in Table 1). On an oscilloscope at 2 mV/div observe the 10 V_m output through the filter set to 100 Hz. Use the OFFSET control to center the trace on the screen.
4) Now ground the headstage input via a resistor equal to R_o[(1)] in Table 1. 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 pot inside the headstage until there is no shift.

Note 1. For values of 1 GΩ or more it is important to clean the surface of the resistor thoroughly to remove leakage pathways.

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

Note 2. If an external source is connected to the EXT. ME1 AND ME2 COMMAND input, any time the source is non-zero a proportional current will flow in the microelectrode. Many external sources do not put out a true zero voltage when in the OFF state, thus

there may be an unwanted microelectrode 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, or use an isolated external source.

DC Removal

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 actual microelectrode potential might be 200 mV (though the experimenter does not see this potential because of the offset compensation). To compensate several pF 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 it. 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 microelectrode 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 Axoclamp-2B called a constant current source (CCS) controls the voltage across R_o. Ideally, the voltage across R_o is independent of the microelectrode 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 $R_o \times 10^4$.

Other possible factors which 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. This technique is known as bootstrapping. Thus the effective resistance of the input is 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.

Offset Controls

The Offset controls compensate for the junction potentials in the experimental setup.

The offset compensation for the V_2 output works by adding a DC voltage to the output. Therefore, it is called the OUTPUT OFFSET control.

The offset compensation for the 10 V_m and V_1 outputs is performed in the first stage of the recording circuit. This is necessary so that after amplification of the input signal the full range of the sample-and-hold circuitry can be utilized. The ME1 offset compensation should **not** be altered during voltage clamp because the voltage-clamp circuitry will interpret the change in the offset setting as a change in V_m. To remind you of this important characteristic the control is called the INPUT OFFSET.

For both controls, the compensation range is ±500 mV. The no-compensation point is in the middle of the range of the multi-turn dials. Each turn of the dials is approximately 100 mV. The dials can be

locked after setting. Calibrated dials are used for these controls because they have brakes to prevent accidental movement; however, the dial markings are not meaningful.

The normal procedure for using the Offset controls is to zero the voltmeter readings when the microelectrode is outside the cell. All subsequent readings are then with respect to the potential of the extracellular solution.

Output Filter

Built-in filters are provided to smooth the 10 V_m and I_m outputs. These are single-pole low-pass filters. Six -3 dB frequencies (f_L) can be selected.

As well as reducing the noise, a filter also slows the rise time of the filtered signal. A single-pole filter converts a step into an exponential. There is no overshoot. The time constant of the exponential is:

$$\tau = \frac{1}{2\pi f_L}$$

The 10 - 90% rise time of the exponential is:

$$t_r = 2.2\tau.$$

The six available f_L's and the corresponding τ's and t_r's are given in Table 4.

TABLE 4

f_L (kHz)	0.1	0.3	1	3	10	30
τ (μs)	1600	530	160	53	16	5.3
t_r (μs)	3500	1200	350	120	35	12

High-Order Low-Pass Filters for Low-Noise Recordings

The "order" of a filter refers to the number of poles (RC sections). For example, a third-order filter has three poles. Each pole attenuates the high-frequency noise at 20 dB/decade.

During TEVC the current noise increases at +20 dB/decade above a frequency determined by the membrane time constant (Finkel & Gage, 1984). To adequately limit this noise the external filter used for data display and storage should be at least 2nd order and preferably 3rd or 4th order.

Rise Time of High-Order Filters

As a rule of thumb it can be noted that for low-pass multiple-pole filters having less than 10% overshoot, the 10-90% rise time is within a few percent of t_r in a single-pole filter having the same -3 dB frequency.

However, the frequency specified for some commercially available (non-Axon) multiple-pole low-pass filters is the -3 dB frequency of the component lower-order filters instead of being the -3 dB frequency of the complete filter. Before using these filters it is advisable to check the 10-90% rise time of a step signal applied to the input.

Note on Ultimate Rise Time

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

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

Phase Lag

A voltage clamp is a negative-feedback circuit and as such it requires a 90° phase shift within the circuit. Ideally this phase shift is supplied by the capacitance of the membrane. In practice, membranes introduce significantly less than 90° phase shift (see discussion by Finkel & Gage, 1984).

The frequency response of the voltage-clamp circuit can be modified by the PHASE LAG controls. The voltage-clamp circuit can thereby be adjusted to compensate for the nonideal phase response of real membranes.

The controls are in two parts; a potentiometer to shift from 0.01 to 1.0, and a 5-position multiplier switch .

Phase lag **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. In the extreme lag position the phase-control circuit introduces pure lag.

The five settings (0.01, 0.1, 1, 10 and 100) of the MULTIPLIER switch are used to change the maximum lag. In some instances no phase lag is required (*e.g.,* with an RC cell model the best TEVC or dSEVC will be achieved when **no** phase lag is used). If this is so, the MULTIPLIER switch should be switched to the OFF position.

Use

The PHASE LAG can be used during voltage clamp to compensate for the frequency characteristics of membranes which are not well modeled by a parallel resistance and capacitance. Both the membrane voltage and current step responses should be improved by using the PHASE LAG. If only the membrane voltage step response is improved it is likely that there is a resistance (R_s) in series with the membrane. See the *Series Resistance* section for a discussion of this problem.

In some cases using some phase lag will reduce the current noise during voltage clamp. See the discussions on each type of voltage clamp mode for more details.

Series Resistance

A resistance (R_s) in series with the membrane can arise in a number of different ways. The voltage-recording microelectrode (ME1) records the voltage across R_s and the membrane resistance, R_m, thus the recorded membrane potential is in error due to the IR voltage drop across R_s. In addition, R_s limits the maximum rate at which the membrane capacitance can be charged.

Sources of R_s depend on the recording mode. In continuous single-electrode voltage clamp (cSEVC) mode, R_s would mainly be due to the resistance of the suction electrode. In discontinuous single-electrode voltage clamp (dSEVC) mode, R_s would be due to a slow microelectrode response. In two-electrode voltage clamp mode (TEVC) current does not flow through the recording electrode; thus the

microelectrode resistance does not contribute to the R_s error. However, there are three main contributors to R_s in TEVC mode. (1) The resistance between the membrane surface and the bath ground electrode. This will include the resistance of the bath solution which is generally not significant. It will also include the resistance between the bath solution and the membrane surface. This can be significant where there is extensive infolding of the surface membrane, for example. (2) The resistance of the agar filled ground electrode. This is usually the largest single contributor. (3) The resistance of the grounding wire and Ag/AgCl pellet. This is generally not significant.

Whatever your application it is good practice to minimize the sources of R_s where possible, but before you take additional steps to reduce R_s it is worthwhile to determine the potential magnitude of the problem in your system. Once you have an idea as to the maximum currents flowing you will have a better idea as to the maximum tolerable R_s value. R_s can be approximately calculated from the peak of the capacitive transient (I_p) during a voltage step: $R_s = V_{step} / I_p$. For this calculation to be accurate the voltage clamp gain must be high and the output filtering must not attenuate the response.

For detailed discussion of the series resistance problems and their solutions in cSEVC and dSEVC see the respective clamp modes, and for TEVC see *Bath Error Potentials* on p. 59.

Unity-Gain Recording — Third Point

In normal operation both ME1 and ME2 can be used for unity-gain recording and current-passing. A third point in the preparation can be recorded from if virtual-ground current measurement is not being used. To do so, a unity-gain headstage (HS-2) is plugged into the BATH-CLAMP PROBE (I) connector on the rear panel. The voltage recorded appears on the I_{BATH} output or the current meter, if set to I_B. No current can be passed via the HS-2 headstage used in the BATH-CLAMP PROBE (I) connector. When plugged into the BATH-CLAMP PROBE (I) connector the input capacitance of the unity-gain headstage is approximately 4 pF.

Virtual-Ground Current Measurement

A Virtual-Ground headstage can be used to ground the preparation bath. Connect the headstage to the BATH-CLAMP PROBE (I) input. All of the current flowing into the Virtual-Ground input is measured and a voltage proportional to the current is provided at the I_{BATH} output. The output gain is 100 mV/nA when the virtual-ground attenuation (VG) is x0.1, 10 mV/nA when VG is x1, 1 mV/nA when VG is x10, and 0.1 mV/nA when VG is x100.

Table 5
Current recording ranges for virtual ground headstages

	R_f (MΩ)	Full scale range	Conversion
VG-2-x0.1	100	±0.1 µA	100 mV/nA
VG-2-x1	10	±1 µA	10 mV/nA
VG-2-x10	1	±10 µA	1 mV/nA
VG-2A-x100	0.1	±100 µA	0.1 mV/nA

A Virtual-Ground headstage is not required for normal use of the Axoclamp-2B because built-in current-measurement circuits are provided for each microelectrode. However, in TEVC mode the

current output of the Virtual-Ground headstage has slightly less high-frequency noise than the output of the built-in current-measurement circuit.

The Virtual-Ground circuit measures all currents into the preparation bath. Thus if an ionophoretic microelectrode is to be used, it must have its own separate current return microelectrode (adding yet another electrode to the bath), otherwise the ionophoretic current will appear in the clamp current. A more insidious problem arises because the bath effectively becomes a very sensitive antenna and will pick up very low levels of power line radiation which will be evident as line frequency noise in the current signal. Saline-filled tubing acts as an excellent antenna. To prevent them carrying hum, long saline-filled tubes should have the saline pathway broken by an air-filled drip near the preparation.

Another potential source of error using this current-measurement technique is the fact that there will be direct capacitive coupling between the current-passing microelectrode and the ground electrode. This current bypasses the cell membrane but appears on the current signal. Generally this will not pose a significant problem, provided that the current-passing microelectrode is carefully shielded.

More complete instructions for using these headstages are provided in the manual provided with the VG series of headstages.

10 V_m And I_m Outputs

The 10 V_m output provides ten times the membrane potential (V_m). It is derived from the potential (V_1) recorded by ME1. Initially V_1 is amplified, then, depending on the operating mode, one of two techniques is used to derive the 10 V_m signal from the amplified V_1 signal. In BRIDGE mode, the bridge balance technique is used to counter the effect of voltage drop (IR voltage drop) across ME1 during current passing so that only the membrane potential measured at the tip is passed to the 10 V_m output. In discontinuous current clamp (DCC) or discontinuous single-electrode voltage clamp (dSEVC) mode, samples of the amplified V_1 signal are taken after the decay of the IR voltage drop across ME1 due to the previous current pulse. Only the sampled values are passed to the 10 V_m output.

The maximum recording range of the 10 V_m output is ±600 mV referred to the input. This range is centered on the zero value set by use of the INPUT OFFSET control. In BRIDGE mode this range includes the IR drop even though the IR drop may not be seen because the Bridge is correctly balanced. The full ±600 mV input-referred range is available in DCC and dSEVC modes irrespective of the current.

The I_m output is proportional to the membrane current. In BRIDGE, cSEVC and TEVC modes it is the continuous electrode current. In DCC and dSEVC modes, I_m is found by sampling the current during the current-passing period and multiplying by the duty cycle.

REFERENCE GUIDE: THEORY OF RECORDING MODES

Bridge Mode

In BRIDGE mode the microelectrode voltages are monitored continuously, and continuous currents can be injected down ME1 or ME2.

Associated with the current flow (I) in a microelectrode is a voltage drop across the microelectrode which depends on the product of the current and the microelectrode resistance (R_e). This unwanted IR voltage drop adds to the recorded potential. The BRIDGE control can be used to balance out this voltage drop so that only membrane potential is recorded. The term "Bridge" refers to the original Wheatstone Bridge circuit used to balance the IR voltage drop and is retained by convention even though the original circuitry has been replaced by operational amplifier techniques.

The particular setting required to balance the Bridge is a measure of the microelectrode resistance, and the microelectrode resistance can be read from the dial setting.

In cSEVC mode the BRIDGE potentiometer compensates electrode IR voltage drop at one-tenth sensitivity.

It is sometimes useful to inject a brief small current pulse at the start of each sweep of data collection in order to continually check the BRIDGE setting during the course of an experiment.

Continuous Single-Electrode Voltage Clamp (cSEVC) Mode

To implement a continuous single-electrode voltage clamp (cSEVC; also known as whole-cell patch clamp), a blunt, low-resistance pipette is fused by suction to the surface of the membrane. The patch of membrane within the tip of the pipette is ruptured by one of a variety of techniques. The electrolyte in the patch pipette is thus in electrical continuity with the interior of the cell. It is equivalent to an extremely low resistance (approximately 1-10 MΩ) intracellular micropipette.

In cSEVC, the voltage at the *top* of the patch pipette is controlled by a voltage-clamp circuit. The same pipette is used simultaneously for voltage recording and for current passing, as shown in the block diagram of Figure 20. The voltage (V_1) recorded by the pipette buffer (A1) is compared in a high-gain differential amplifier (A2) to a command potential (V_c). The output of A2 acts to keep the difference at its input (ε) very small. Hence, V_1 is clamped equal to V_{cmd}.

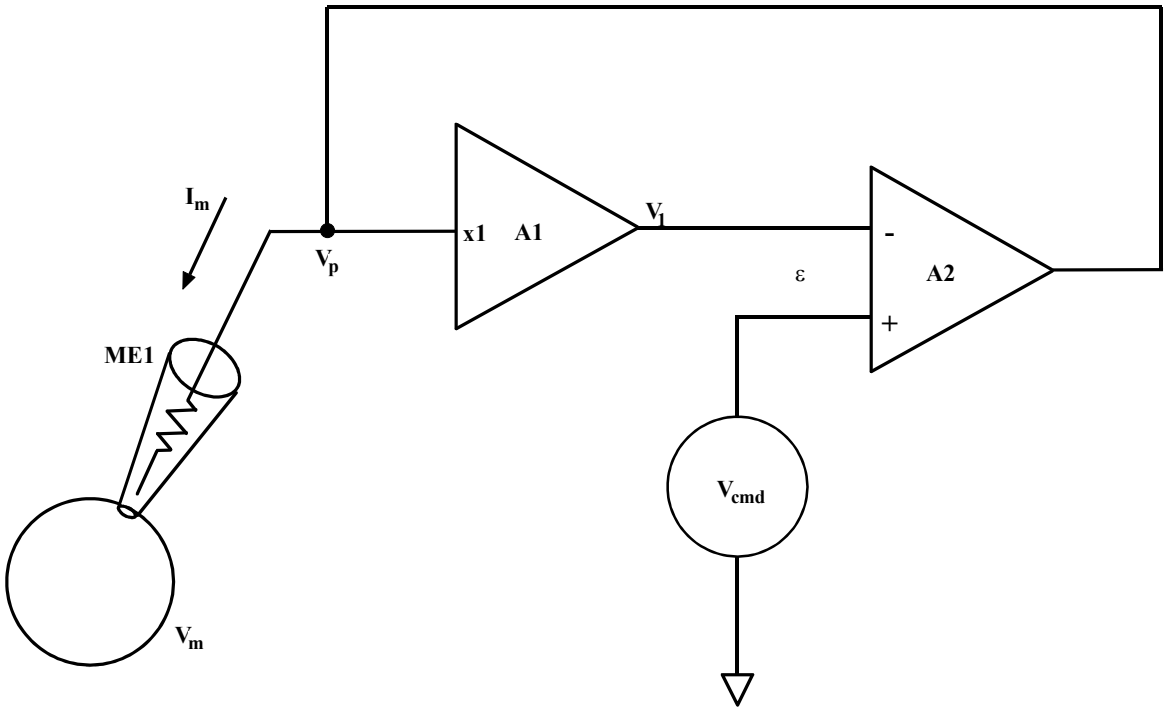


Figure 20. Simplified schematic of cSEVC

The voltage, V_p, recorded at the top of the patch pipette is the sum of the membrane potential, V_m, which it is desired to control, and the current-induced voltage drop across the pipette. It is important to realize that this is quite different than the situation in TEVC or dSEVC. In both of these latter cases, the voltage at the *tip* of the voltage-recording micropipette, *i.e.*, V_m, is controlled (remember, in dSEVC, time-division multiplexing effectively yields two micropipettes; the voltage-recording micropipette and the current-passing micropipette).

Axoclamp-2B cSEVC Mode Compared to a Patch-Clamp Amplifier

Although the Axoclamp-2B's cSEVC is similar to whole-cell clamping using a patch-clamp amplifier, the implementation is very different.

In the patch-clamp amplifier the voltage-clamp circuit is a current-to-voltage converter located in the headstage. In contrast, the Axoclamp-2B cSEVC mode uses a headstage that is a general-purpose unity-gain buffer and the voltage-clamp circuit is located in the main unit.

This difference can be significant. In the patch-clamp amplifier less circuitry is involved and thus nonidealities of the electronics have less effect. Thus, for fast events the patch-clamp amplifier is considerably better.

On the other hand, for slow and moderate speed events the performance of the two instruments becomes comparable. While electrically the circuitry works better when the current-to-voltage converter is located in the headstage, in practice it turns out that similar noise and step responses can be achieved in many cells using the cSEVC mode of the Axoclamp-2B.

Pipette Capacitance Compensation

When the command voltage is stepped, a large amount of current flows into the pipette capacitance during the transition from one potential to the next. This is reduced by setting the CAPACITANCE NEUTRALIZATION control (see *Capacitance Neutralization And Input Capacitance* in this chapter). This control and the FAST mag compensation controls of an Axopatch are similar in function. Unlike the patch-clamp amplifier, there are no whole-cell capacitance compensation controls in the Axoclamp-2B.

Series Resistance Compensation

The current through the access resistance, composed of the series resistance of the patch pipette and the residual resistance of the ruptured patch, is often sufficiently large to introduce significant voltage errors. In the ideal experiment, the resistance of the patch pipette in whole-cell experiments would be zero. In this case, the time resolution for measuring membrane currents and changing the membrane voltage would be limited only by the speed of the electronics (typically just a few microseconds).

Series resistance compensation using positive feedback is an attempt to achieve this ideal electronically. Basically, a signal proportional to the measured current is used to increase the command potential. This increased command potential compensates in part for the potential drop across the micropipette. The amount of compensation achievable is limited by two considerations. First, as the compensation level (α) approaches 100%, the increase in the command potential hyperbolically approaches infinity. For example, at 90% compensation, the command potential is transiently increased by a factor of ten ($V_{cmd}/(1 - \alpha)$. Thus at large compensation levels the electronic circuits approach saturation. Second, the current feedback is positive, therefore the stability of the circuit is degraded by the feedback and at 100% compensation the circuit becomes an oscillator. In practice, the oscillation point is much lower than 100% because of non-ideal phase shifts in the micropipette and the cell membrane.

The first problem, saturation of the electronics, could in principle be reduced by using high-voltage (*e.g.,* ±130 V) operational amplifiers, but this approach has not been pursued because these types of operational amplifier have more noise and worse drift than good conventional operational amplifiers. The second problem, stability, can be partially reduced using the phase lag, a variable low-pass filter in the current-feedback loop. The setting of the low-pass filter cutoff frequency is determined empirically. Large percentage compensations can then be used, but these only apply to the currents at bandwidths below that of the filter cutoff. Thus the DC, low and mid frequency series resistance errors can be substantially reduced while the high-frequency errors remain large.

In the Axoclamp-2B operating in cSEVC mode the maximum compensation is usually about 70%, beyond which the system oscillates and destroys the cell. To get a feeling for the magnitude of the errors, assume that compensation of 70% is achieved. Further assume that the access resistance (R_a; the sum of the pipette resistance and the residual resistance of the ruptured patch) is 5 MΩ. After compensation, the effective value of R_a ($R_{a,eff}$) is just 1.5 MΩ. In this case, the error caused by a 10 nA current, which uncompensated would cause a 50 mV voltage error, is reduced to 15 mV by the compensation. Clearly, the cSEVC technique cannot be used to record large currents, and even for modest whole-cell currents care must be taken to compensate for the series resistance and then correctly interpret the residual error. The dSEVC technique should be considered as an alternative to the cSEVC technique when the access resistance is too large.

Advantages of Whole-Cell Recording With a Patch Pipette

The advantages of whole-cell recording with a patch pipette compared to using a micropipette stem primarily from the lower access resistance achieved with the patch pipette. The lower access resistance gives several advantages, foremost among them, lower recording noise and better voltage control of the cell's membrane potential. The lower recording noise allows the detection of currents that are not easily detected using micropipettes. This has been used in several studies to record very small synaptic currents resulting either from minimal electrical stimulation, or occurring spontaneously in brain tissues.

Figure 21 contrasts recordings from brain slices in BRIDGE mode using micropipettes and in cSEVC mode using patch pipettes. Sample records show spontaneous inhibitory postsynaptic potentials (IPSPs) or currents (IPSCs) recorded in hippocampal CA1 pyramidal cells by the two different methods. In both cases, the hippocampal slices were bathed in glutamate antagonists (10 µM CNQX, 50 µM AP-5) to block excitatory synaptic transmission, and in tetrodotoxin (1 µM) to block action potential-dependent release. Since these recordings are made in the presence of tetrodotoxin (TTX), they presumably arise from the spontaneous quantal release of the inhibitory transmitter GABA from interneuronal terminals.

On the left are IPSPs taken in BRIDGE mode using a micropipette. The micropipette was filled with 3 M KCl, its resistance was approximately 80 MΩ, and the cell's input resistance was approximately 50 MΩ. On the right are IPSCs taken in cSEVC mode using a patch pipette. The main electrolyte in the patch pipette was 100 mM KCl. The electrode resistance was approximately 2 MΩ and the cell's input resistance was greater than 200 MΩ.

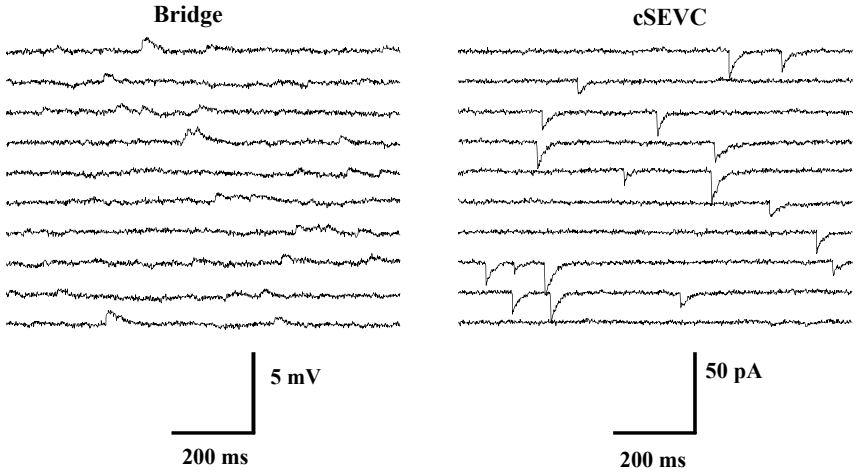


Figure 21. Recordings from brain slices in bridge mode and cSEVC mode

Note that although the spontaneous IPSPs can be detected using a micropipette, they are small, poorly resolved from the noise, and have slow rise-times. In contrast, in the cSEVC recording, the IPSCs have fast, well-resolved rise times and are more clearly distinct from the noise. With a typical amount of recording noise, the smallest spontaneous IPSP that can be resolved well enough to get a good amplitude measurement is about 1 mV. With a typical 50 MΩ input resistance, this corresponds to a synaptic current of about 20 pA. In cSEVC mode, it is possible to get good amplitude measurement on events approaching 2 pA in size. Thus, the use of the cSEVC technique provides an approximately ten-fold improvement in resolution of these synaptic events.

Macropatch Technique

The Axoclamp-2B in cSEVC mode can be used to clamp macropatches. Headstages with gains of x0.1 and the x0.01 are capable of handling peak currents of 100 nA and 10 nA, respectively.

This technique may be especially useful if the membrane capacitance limits the time resolution of rapidly activating or inactivating voltage-dependent channels studied with the two-electrode voltage clamp. Such a situation is likely to arise in *Xenopus* oocytes expressing rapidly gating channels, such as sodium channels or "A" type potassium channels. 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 measure the kinetics of rapidly gating channels is to use a patch-clamp configuration 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 the cSEVC mode 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 clamped 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 amplifier must be able to fully compensate for the capacitance of the patches. The compensation range of the x0.01M headstage is 0-22 pF and that of the x0.1L is 0-8 pF. Thus, for membrane patches with capacitance greater than 8 pF, the x0.01M headstage must be used.

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.

Discontinuous Current Clamp (DCC) Mode

In Discontinuous Current Clamp (DCC) mode, ME1 is cyclically used to pass current. The voltage recorded at the tip of ME1 is memorized by a sample-and-hold circuit in between each current-passing period after all transient voltages due to current passing have decayed. Thus the membrane potential can be recorded independently of the IR voltage drop across the microelectrode. The advantage of DCC mode compared with BRIDGE mode is that it is tolerant of small changes in microelectrode resistance. The disadvantage is that DCC mode is noisier than BRIDGE mode. During DCC mode ME2 is still in BRIDGE mode and can be used for continuous current passing.

The principles of operation are outlined in the block diagram and timing diagram of Figure 22.

The voltage recorded by the microelectrode (V_1) is buffered by a unity-gain head stage (A1). Assume that V_1 is exactly equal to the instantaneous membrane potential (V_m). Switch S2 briefly closes thereby enabling the voltage on the holding capacitor (C_H) to charge up to the value of V_m. S2 opens again after the "sample" period and V_m is held by C_H. A buffer amplifier (A2) interfaces C_H to the recording apparatus. This switch, capacitor and buffer amplifier arrangement constitute an analog memory known as a sample-and-hold amplifier.

Immediately after the sample period, the current injection period begins when switch S1 changes over from the zero volts position to the current-command voltage (V_{cmd}) position. This connects V_{cmd} to a differential amplifier (A4) arranged so that its output is $V_1 + V_{cmd}$. The voltage appearing across R_o is exactly equal to V_{cmd} thereby forcing the current (I_o) into the microelectrode to be equal to V_{cmd}/R_o. Amplifiers A4 and A1 and resistor R_o constitute a controlled-current source (CCS) which injects a current into the microelectrode directly proportional to the voltage at the input of the CCS irrespective of the resistance of the microelectrode or the voltage at its tip.

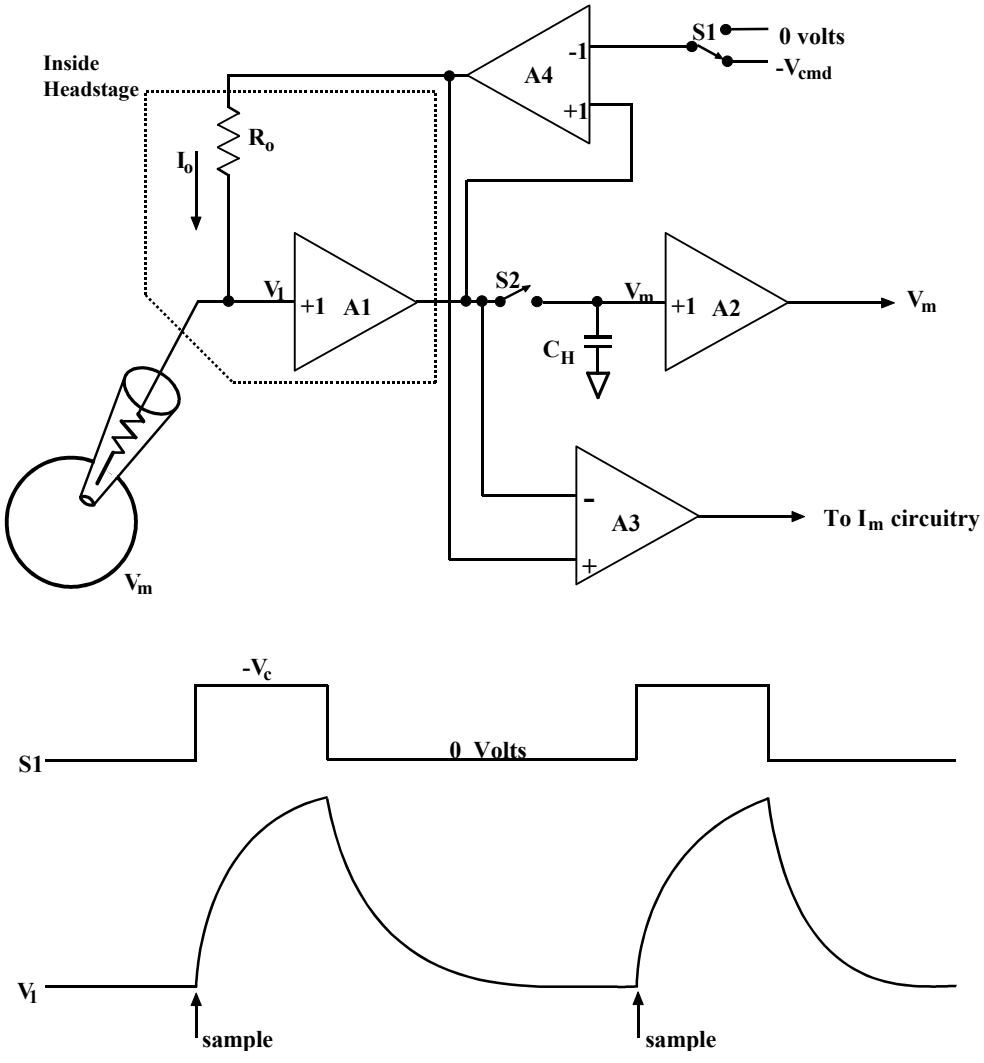


Figure 22. DCC Mode block diagram and timing diagram

During the current-injection period a square pulse of current proportional to V_{cmd} is injected into the microelectrode. Because of this current V_1 rises. The rate of rise of V_1 is limited by the parasitic effects of capacitance through the wall of the glass microelectrode to the solution, and capacitance at the input of the buffer amplifier. The final value of V_1 reached consists mostly of the IR voltage drop across the microelectrode resistance. Only a tiny fraction of V_1 consists of the membrane potential recorded at the tip.

After 30% of one cycle has elapsed, the voltage-recording period begins when S1 changes back to the zero volts position. Passive decay occurs because the input of the CCS is zero volts and thus its output current is zero. Sufficient time must be allowed during the voltage-recording period for V_1 to decay to within a millivolt or less of V_m. At the end of the passive decay period S2 is again briefly closed and a new sample of V_m is taken to begin a new cycle.

The actual voltage used for recording purposes is the sampled voltage. The 10 V_m output is the sampled membrane potential. The V_1 CONT. output is the instantaneous microelectrode voltage.

The instantaneous current into the microelectrode is monitored by a differential amplifier (A3). The output of A3 is taken to an averager (not shown) which samples, smoothes and scales the current pulses and this average value is available on the I_m output.

During DCC mode the input to the CCS and the output of the ME1 current monitor are automatically scaled so that they represent the true membrane current even though the instantaneous current flows for only 30% of the time.

The cycling (sampling) rate must be chosen so that there are ten or more cycles per membrane time constant. This enables the membrane capacitance to smooth the membrane voltage response to the current pulses.

Discontinuous Single-Electrode Voltage Clamp (dSEVC) Mode

Although two-electrode voltage clamping is faster, the Axoclamp-2B allows **very fast** discontinuous single-electrode voltage clamping. In a model cell (10 MΩ//1 nF) using a 10 MΩ resistor to model the microelectrode, the 10 - 90% rise time is only 100 µs. In a real setup the response speed is limited by the microelectrode characteristics, but membrane potential rise times (without overshoot) of less than 1 ms have been regularly achieved in a variety of cell types. The discontinuous single-electrode voltage clamp mode can be used with either micropipettes or patch pipettes.

In discontinuous single-electrode voltage clamp (dSEVC) mode the tasks of voltage-recording and current-passing are allocated to the same microelectrode. Time-sharing techniques are used to prevent interactions between the two tasks. The principles of operation have been published (Brenneke & Lindemann, 1974; Wilson & Goldner, 1975; Finkel & Redman, 1984) and are outlined in the block diagram and timing diagram of Figure 23, and in the following discussion.

A single microelectrode (ME1) penetrates the cell and the voltage recorded (V_1) is buffered by a unity-gain headstage (A1). To begin the discussion assume that at this moment V_1 is exactly equal to the instantaneous membrane potential (V_m). A sample-and-hold circuit (SH1) samples V_m and holds it for the rest of the cycle.

The sampled membrane potential is compared with a command voltage (V_{cmd}) in a differential amplifier (A2). The output of this amplifier becomes the input of a controlled-current source (CCS) if the switch S1 is in the current-passing position. The gain of the CCS is G_T. The CCS injects a current into the microelectrode which is directly proportional to the voltage at the input of the CCS irrespective of the resistance of the microelectrode.

The period of current injection is illustrated at the start of the timing waveform. S1 is shown in the current-passing position during which a square pulse of current is injected into the microelectrode. Because of this current, V_1 rises.

The rate of rise is limited by the parasitic effects of the capacitance through the wall of the glass microelectrode to the solution, and the capacitance at the input of the buffer amplifier. The final value of V_1 mostly consists of the IR voltage drop across the microelectrode due to the passage of current I_o through the microelectrode resistance R_e. Only a tiny fraction of V_1 consists of the membrane potential recorded at the tip.

S1 then switches to the voltage-recording position. When the input of the CCS is zero volts, its output current is zero and V_1 passively decays. During the voltage-recording period V_1 decays asymptotically towards V_m. Sufficient time must be allowed for V_1 to reach within a millivolt or less of V_m. This requires a period of up to nine electrode time constants (τ_e). At the end of the voltage-recording period a new sample of V_m is taken and a new cycle begins.

The actual voltage used for recording purposes is the sampled voltage. As illustrated in the bottom timing waveform the sampled value of V_m moves in small increments about the average value. The difference between V_m(average) and V_{cmd} is the steady-state error (ε) of the clamp which arises because the gain (G_T) of the CCS is finite. The error becomes progressively smaller as G_T is increased.

The duty cycle used in dSEVC mode is current passing for 30% of each cycle, and voltage recording for 70% of each cycle.

The cycling rate (sample rate) must be chosen so that there are ten or more cycles per membrane time constant. This enables the membrane capacitance to smooth the membrane voltage response to the current pulses.

When optimally adjusted, the circuit enables the first steady-state measurement of voltage to be taken 1 to 2 cycle periods after the onset of a membrane conductance change or a change in the command voltage.

Two controls not shown in the figure are the ANTI-ALIAS FILTER and the PHASE LAG. The ANTI-ALIAS FILTER is a single-pole filter between the output of the unity-gain headstage (A1) and SH1 (see Figure 17). It can be used to reduce noise at a given sampling frequency. The output of the ANTI-ALIAS FILTER can be observed on the MONITOR output. In practice it is this voltage, not V_1, which has to decay to V_m before a sample is taken. The PHASE LAG control alters the frequency response of the differential amplifier (A2). It can be used to compensate for the complicated frequency characteristics of a real cell.

The Gain control alters G_T. Its operating range is (100 x H) nA/mV.

While ME1 is used dSEVC mode it is still possible to independently use ME2. For example, ME2 could be used for recording from and stimulating other cells which make connections to the cell being voltage-clamped.

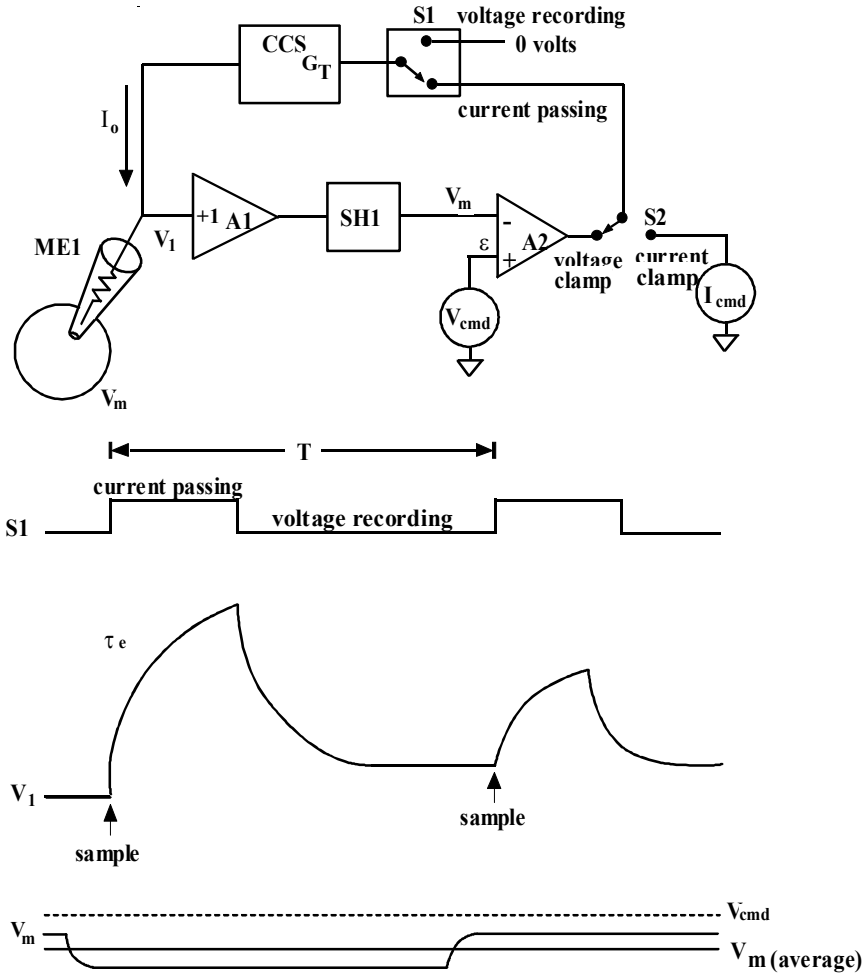


Figure 23. dSEVC block diagram and timing waveforms

Minimum Sampling Rate And Maximum Gain

If the sampling rate is too slow the dSEVC will become unstable. This is because the long current-passing period charges the membrane potential past the desired potential before the clamp has an opportunity to take a new sample of potential and adjust the current accordingly. The larger the cell membrane capacitance (C_m) the slower the sampling rate (f_s) that can be used for a given average gain (G). The stability criterion is (see Brenneke & Lindemann, 1974; Finkel & Redman, 1984):

$$0 < \frac{G}{C_m f_s} < 2$$

For critical damping we require $\frac{G}{C_m f_s} = 1$

Thus for a given G, if C_m is small f_s must be large.

As an example, if G = 1 nA/mV and C_m = 100 pF, then f_s must be 10 kHz for critical damping. If f_s is less than 10 kHz in this example, the step response will overshoot and at 5 kHz the clamp will oscillate destructively.

If the sampling rate in this example cannot be as great as 10 kHz because the microelectrode response is too slow, then a lower value of G will have to be used to maintain stability.

Clamp Error

With finite gains in the voltage-clamp circuit V_m does not quite follow V_c. The error is $\varepsilon = V_{cmd} - V_m$.

Similarly, if V_{cmd} is constant and the cell membrane conductance changes, then there is an error in the measurement of the current underlying the conductance change. This error is similar in percentage to the voltage error.

Usually the gain of the voltage-clamp circuit can be increased so that ε is 10% or less. The percentage error depends on the frequency of the command signal or of the conductance change. It is smallest for slow signals and DC, and largest for the fastest signals. Thus very fast transients (such as the rising phase of synaptic currents) will be clamped less well than slower transients (such as the decay phase of synaptic currents).

Gain

The clamp gain during dSEVC mode is given in nA/mV. This refers to how many nanoamperes the output current will change by for each millivolt of difference between V_m (the membrane potential) and V_{cmd} (the command potential). The value indicated on the front panel is the average value (G). The average value depends upon the instantaneous gain during the current-passing period (G_T) and upon the duty-cycle.

Series Resistance, R_s

In dSEVC mode the effect of series resistance, R_s, is to slow the microelectrode response. Thus, the current does not completely decay to baseline before the voltage measurement. Since the potential recorded by the membrane potential is the sum of the voltage across R_s and the membrane resistance, R_m, there is an error in the membrane potential recorded. The added consequence is that R_s limits the maximum rate at which the membrane can be charged. To eliminate R_s altogether, watch the MONITOR output and make sure the transient decays completely before the next sample is taken.

Advantages of a Patch Pipette

It turns out that a patch pipette is ideal for dSEVC. That is, when R_e (microelectrode resistance) is very small, its time constant is fast. In addition, the magnitude of the voltage transient across the microelectrode for a given current is proportional to R_e and therefore small when R_e is small. This double advantage of low R_e values means that the dSEVC can be cycled very rapidly without introducing a sampling error.

dSEVC and cSEVC Compared

Single-electrode voltage clamp (SEVC) mode can be used to implement a whole-cell patch voltage clamp in one of two modes of operation, continuous or discontinuous. Each of these modes has its own advantages for recording.

The continuous SEVC (cSEVC) mode is the more simple to operate and gives the lower noise recording of membrane current. Because it records current continuously, it also avoids some of the problems created by the charging and discharging of the microelectrode capacitance during the duty cycle of discontinuous clamp. Problems with circuit instability ("ringing") are less severe in cSEVC mode. The major shortcoming of the cSEVC mode is that command voltage, rather than membrane voltage, is monitored by the voltage clamp, so it is often difficult to assess the quality of control of the cell's membrane potential. Put differently, when the currents being clamped are modest or large in size (> 5 nA) there will be a significant error in the clamp potential due to the uncompensated series resistance. However, for small currents, the error due to the uncompensated series resistance can generally be made negligible, and the cSEVC mode is very attractive.

In discontinuous SEVC (dSEVC) mode, the Axoclamp-2B repetitively cycles between current passing (through the electrode) and voltage-measuring, at a frequency that is set by the user. The current-induced voltage drop across the microelectrode is allowed to decay completely before each sample of the membrane potential is taken. Thus, dSEVC offers the main advantage that the clamp measures actual membrane voltage, so that the quality of voltage control can be observed directly. The dSEVC is generally superior to cSEVC when the currents being clamped are modest or large in size (> 5 nA), because if the sampling rate is correctly chosen and the capacitance compensation correctly set, there is *no* error due to series resistance. For small current, the dSEVC mode is less attractive because it is harder to set up and because it is noisier.

Current and potential during dSEVC and cSEVC using the same patch pipette model are compared in Figure 24. In Figures 24A-C are shown the recordings obtained with cSEVC mode and in Figures 24D-F those obtained with dSEVC.

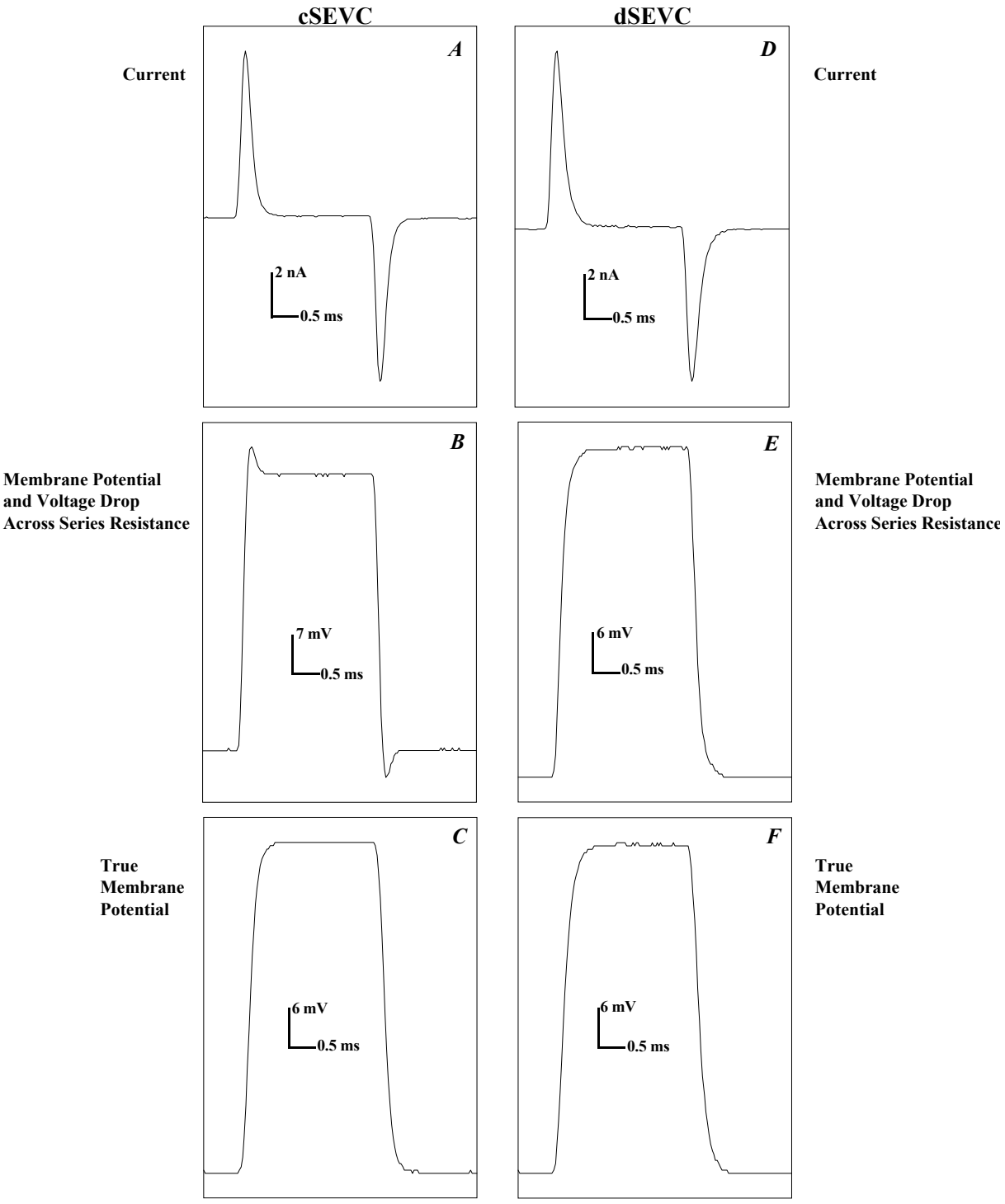


Figure 24. Comparison of current and potential during dSEVC and cSEVC using the MCW-1U model cell

The settings for each type of SEVC are given in Table 6.

Table 6

	cSEVC	dSEVC
GAIN	17 nA/mV	0.7 nA/mV
ANTI-ALIAS FILTER	Off	0.5 µs
PHASE LAG	1.2 ms	none
SAMPLE RATE	Off	20 kHz

The most significant difference in the setup besides the clamping mode used is that no phase lag is used in the dSEVC. Since the IR drop across the patch pipette is not sampled, the recorded potential during dSEVC (Figure 24E) has the same time course as the membrane current (Figure 24D) and the true membrane potential recorded by an independent patch pipette (Figure 24F). The major disadvantage of the dSEVC mode is the additional current noise. The peak-to-peak current noise in dSEVC mode (Figure 24D) is ≈1.5 times greater than that in cSEVC mode (Figure 24A).

Noise in DCC and dSEVC Modes

The noise inherent in discontinuous single-electrode clamps (discontinuous current clamp or discontinuous single-electrode voltage clamp) is four or more times greater than the noise in continuous electrode clamps (bridge current clamp or two-electrode voltage clamp) when the discontinuous electrode clamps are adjusted for the same dynamic response and accuracy as the continuous electrode clamps.

There are two major reasons for this inherent deterioration in noise performance.

The first is due to capacitance neutralization. A fundamental property of all capacitance neutralization circuits is that they introduce noise in excess of what is contributed by the thermal noise of the recording microelectrode and the input noise of the buffer amplifier. The excess noise becomes progressively larger as the microelectrode time constant is reduced. In discontinuous systems the microelectrode time constant must be deliberately reduced more than in continuous systems so that after a current pulse the microelectrode voltage will decay to V_m within the time allotted for passive recording. The excess noise due to optimizing the capacitance neutralization can vary from a factor of about two in a system where primary efforts have been taken to keep the input capacitance low, to much larger factors in systems where large amounts of capacitance-to-earth and capacitance-to-shield are tolerated.

The second major reason for the deterioration in noise performance of discontinuous single-electrode clamps has to do with the sampling process. As discussed in the section on the Anti-Alias Filter, sampling processes alias the noise in the input signal spectrum into a larger-magnitude spectrum confined to a bandwidth equal to half of the sampling rate (f_s). The normal procedure used in digitizing systems to avoid aliasing is to reduce the bandwidth of the input signal to $f_s/2$ or below. This is not possible in discontinuous single-electrode clamping because reducing the bandwidth of the microelectrode increases the time constant and therefore prevents adequate settling. The amount of aliased noise depends in part on the duty cycle used in the discontinuous clamp. The 30% duty cycle used in the Axoclamp-2B has been chosen to give a good compromise between aliased noise and dynamic performance (Finkel & Redman, 1984b). With this duty cycle the increase in noise due to aliasing is a factor of about two.

The two contributions to noise discussed above lead to a factor of four or more deterioration in noise. To keep the deterioration as small as this the experimenter should try to do the following: (1) Keep the real value of C_{in} as small as possible so that only minimal capacitance neutralization must be used. (Avoid using coaxial cable to connect the microelectrode to the headstage.) (2) Either increase the Anti-Alias Filter setting at a given cycle rate, or increase the cycle rate at a given setting of the Anti-Alias Filter, so that the amount of aliased noise is minimized.

Finally, the amount of noise recorded can be reduced to some extent by using as much output filtering as possible. However, the output filtering should never be increased to the extent that dynamic information (*e.g.,* rise time) is lost. Usually, output filtering at $f_s/10$ is a good compromise. The best way of reducing noise in the records is by averaging repetitive responses. This well-known procedure reduces the noise by the square root of the number of averages without affecting the time course of the signal.

Although the noise performance of discontinuous single-electrode voltage clamps is poorer than that of the two-electrode voltage clamp, the single-electrode technique is extremely rewarding because it allows voltage clamping to be performed in preparations where two-electrode voltage clamping is not feasible such as when the cell is buried beneath many layers of other cells. In addition, the signal-to-noise ratio in many preparations during discontinuous single-electrode voltage clamp is, despite the above considerations, adequate for data to be analyzed without averaging.

TEVC Mode

In two-electrode voltage clamp (TEVC) mode the Axoclamp-2B acts as a conventional voltage clamp with an output compliance of **±130 V**. ME1 is the voltage-recording microelectrode and ME2 is the current-passing microelectrode.

The output of the clamp is a voltage source which is connected to ME2. The voltage-clamp GAIN control determines the steady-state accuracy and the response speed. The GAIN control is marked in units of V/V. This refers to how many volts the output will change for each volt of difference between V_m (the membrane potential) and V_{cmd} (the command potential). For example, when the gain is at its maximum value of 10,000 V/V, a 100 µV difference between V_m and V_{cmd} would cause the output to shift by 1 V. If the resistance of ME2 was 10 MΩ there would be a current shift of 100 nA.

Several other controls affect the voltage-clamp response. The PHASE LAG introduces phase lag in the voltage-clamp amplifier. The HOLDING POSITION control shifts the clamped membrane potential. The CAPACITANCE NEUTRALIZATION setting of ME1 affects the voltage-clamp response. The CAPACITANCE NEUTRALIZATION setting of ME2 affects the current monitoring circuit at high frequencies and also has a small effect on the voltage-clamp response. The ANTI-ALIAS FILTER slows the microelectrode response and usually is not be used in TEVC mode. However, it may be useful (see *Anti-Alias Filter* in this chapter).

The best settings of the voltage-clamp parameters are found by setting up the best possible response to a step change in V_c. Usually, the ability of the voltage clamp to follow a step change in command is identical to the ability of the voltage clamp to follow a step change in membrane conductance (Finkel & Gage, 1985).

General Considerations

It is worthwhile to examine the pros and cons of the TEVC technique. While the TEVC technique is more powerful than techniques using single microelectrodes, it is considerably more complicated and difficult to implement. However, the extra effort will be well rewarded. If you are unfamiliar with the theory behind TEVC systems then Taylor (1991) gives a good general overview with a number of useful references. Finkel and Gage (1985) provides a more thorough discussion of the theoretical aspects.

The main advantages of the TEVC technique are:

(1) High current-passing capacity — for larger cells single-electrode clamps will not work adequately, if at all. An Axoclamp-2B in TEVC mode can supply current in the mA range. A common application for two electrode voltage clamps is recording currents in amphibian oocytes.

(2) Excellent time resolution — the rise time for a voltage step with a TEVC can be a factor of 3 or more faster than a single-electrode clamp implemented using similar microelectrodes. A high frequency response might be required to record very rapid tail currents or gating currents associated with voltage-gated channels, for example.

(3) Low noise — current noise levels with a TEVC are generally lower than single-electrode voltage clamps implemented using microelectrodes of comparable resistance, even given the higher frequency response of the TEVC.

The major drawbacks to TEVC systems are that two microelectrodes must be applied to one cell and that there is added complexity.

Configuration of the Voltage Clamp System

Before setting up the voltage clamp you will have to decide how you are going to record the membrane potential (V_m) and how you will measure the membrane current (I_m). The decisions you make will be determined by what you want to achieve and the particular characteristics of your preparation and bath. The brief discussions of the various options that follow will help you assess your particular requirements.

Voltage Recording and Series Resistance

The reference point for the voltage recorded by ME1 is the system ground and under ideal conditions a bath ground electrode holds the extracellular solution at a constant voltage relative to the system ground. After ME1 is offset to zero volts in the bath and then applied to the cell, it will provide the voltage clamp circuit with an accurate measure of V_m provided that there is no net current flow. Under normal conditions however, there is current flowing across the membrane and this current will not only produce a voltage drop across the membrane but also across any resistance that is in series with the membrane and the system ground. These extraneous resistances can be lumped together as "series resistance" (R_s). The voltage drop across R_S is called the series resistance error or bath error potential. The voltage drop across R_s causes the bath potential to deviate from the zero current potential by an amount directly proportional to the current flowing. Thus, for example, if you have a series resistance of 2 kΩ and a 5 μA peak current occurs during a voltage step, then the peak membrane voltage error will be 10 mV.

For a detailed discussion of this type of series resistance and the methods used to minimize it, see *Bath Error Potentials* on p. 59.

Selection of the Membrane Voltage Recording Headstage (ME1)

Usually you will use an "L", or low-noise type headstage, for the voltage-recording microelectrode, as this will allow for some capacitance compensation to be applied if necessary while taking advantage of the very low noise voltage recording characteristics of these headstages. If you have a very large input capacitance then it may be necessary to use an "M" type headstage, although every effort should be made to minimize the sources of the input capacitance (see *Capacitance Neutralization and Input Capacitance* on p. 64).

The headstages are designed to be compact and robust so that they can be mounted on the micromanipulator as close as possible to the recording point. This strategy greatly reduces the input capacitance. For this reason, the electrode holders are designed to plug directly into the headstage input.

It is recommended that you use the simplest configuration and add more complex features as the need arises. In this case all that is required are two headstages, one for voltage (ME1) and the other for current-passing (ME2). The bath ground electrode is plugged into the ground jack on the ME1 headstage.

The reader is referred to the section on *Headstages* in this chapter for specific recommendations regarding the selection of headstages.

Selection of the Current Recording Method

There are two techniques available for recording current. For almost all applications the built-in current monitor will give excellent performance, however some users may wish to bypass the current sensing resistor and, therefore, will have to measure current using the virtual ground technique. The two techniques are discussed in turn below.

If you are interested in obtaining accurate subtraction of the linear capacitive currents at the make and break of a voltage step, then you must ensure that the current measuring device has enough compliance to fully resolve these large transients. Table 7 summarizes the current passing capability of the HS-2A and the HS-4 headstages.

Table 7
Current passing capacities in TEVC mode

Headstage	R_o (MΩ)	Short-Circuit Current	Conversion
HS-2A-x0.1	100	±1.3 µA	100 mV/nA
HS-2A-x1	10	±13 µA	10 mV/nA
HS-2A-x10	1	±130 µA	1 mV/nA
HS-4	0	±5.0 mA	

Driven cases are current limited by a 10 MΩ resistor to a worst case current of 15 µA.

Series Current Measurement (Internal)

To monitor the clamp current it is recommended that you use the internal current monitor in the current-passing headstage. Advantages are simplicity and the fact that only the clamp current is recorded, and not other currents injected into the chamber from ionophoretic electrodes, for example.

Disadvantages are that the current measuring circuit has slightly more high frequency noise and also loads the output of the voltage clamp. In some circumstances this extra load can affect performance. For example, the x1 headstage has a 10 MΩ resistance in series with the output, and the clamp current is measured from the voltage drop across this resistor. If your current-passing microelectrode resistance is 5 MΩ and the output voltage 130 V, then ≈87 V will drop across the current sensing resistor and only ≈43 V across the microelectrode. The effective gain of the clamp is reduced by 1/3rd and thus the frequency response is compromised (see *Tuning the Voltage Clamp — Fidelity and Stability* on p. 98). This will generally not be a significant problem since the high output compliance of the TEVC simply allows a higher clamp gain to be used. If you are using series current measurement and have headstages with different H values, use the headstage with the higher H value (which has the lower value for the current sensing resistor) for the current-passing microelectrode.

Virtual Ground Current Measurement

To further minimize the chance of saturation an optional relay-switched headstage (HS-4) is available to automatically bypass the current-sensing resistor inside the headstage during TEVC mode. In practice the benefit is minor, since the clamp output compliance is generally sufficient to overcome the small additional load presented by the current sensing resistor.

Because the current-measuring resistor is bypassed, the HS-4 series headstage must be used in conjunction with a virtual-ground current monitor (VG-2). The HS-4 headstage is recommended only when large, ultra-fast voltage steps in big cells must be established.

Advantages are that there is slightly less high frequency noise, and there is no additional load on the clamp output when an HS-4 series headstage is used for the current-passing microelectrode.

Disadvantages are that there is an increase in the complexity of setup since an additional headstage is required. More importantly all currents flowing into the bath are recorded.

Further Information: Consult the *Headstages* p. 69 and *Virtual Ground Current Measurement* p. 79 sections in this chapter and the VG-2 headstage manual before using this configuration.

NOTE: The series current recording technique is safer for the operator than the virtual ground technique combined with an HS-4 series headstage, because R_o (typically 1 MΩ) limits the short circuit current. Nevertheless, users should avoid touching the Ag/AgCl wire or pellet of the current-passing electrode when the instrument is in TEVC mode. The output has a compliance of ±130 V on the Axoclamp-2B!

Tuning the Voltage-Clamp — Fidelity and Stability

The object of tuning the voltage clamp is to maximize the feedback gain. There are two reasons, first the bandwidth of the clamp is strongly dependent on the gain and second, the fidelity, or accuracy, with which the voltage clamp tracks the command potential is dependent on the gain.

Fidelity

The steady-state membrane potential (V_m) after a step change in the command voltage (V_{cmd}) is:

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

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 microelectrode (ME2).

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

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. μ is set by the front-panel GAIN control in the range from about 30 to 10,000. If K were unity, the error would vary from 3 percent down to 0.01 percent. However, K is always less than unity, so the error is worse. If the output micropipette resistance is 90 MΩ and the membrane resistance is 10 MΩ, K is 0.1 and the error is ten times worse than if K were unity. Further, during activation of membrane currents R_m can drop dramatically, and K becomes equal to R_m/R_{e2}. Thus, as a rule of thumb it is desirable to use an output micropipette whose resistance is as low as possible, ideally smaller than the resting membrane resistance.

Step Response and Bandwidth

After a step command, the membrane potential relaxes exponentially towards its new value. For $\mu K >> 1$, 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. For example, if R_{e2} = 10 MΩ, C_m = 1000 pF and μ = 100, the time constant is 100 μs. Stated differently, increasing the clamp gain also increases the bandwidth with which V_m can follow changes in V_{cmd}. The -3 dB frequency of the bandwidth is:

$$f_{-3dB} = \frac{\mu}{2\pi R_{e2} C_m}$$

Stability

An ideal voltage clamp is unconditionally stable. The membrane capacitance provides a 90° phase shift which is required for stability in all negative feedback circuits. Unfortunately, in the real world other factors combine to make the circuit unstable at high clamp gains.

The coupling capacitance (C_x) between the microelectrodes is extremely destabilizing. Values as small as 0.01 pF can lead to oscillation if μ has a magnitude of several hundred or more.

Another destabilizing factor is the non-ideal nature of the membrane. In theory the membrane is simply modeled as a parallel resistor and capacitor. In practice, a distributed model applies. The capacitance elements are themselves non-ideal; they should be modeled by an ideal capacitor with a series resistance component. For real membranes, the phase shift at high frequencies is less than 90°. In the Axoclamp-2B, a phase-shift control is included to allow the user to empirically add some phase lag to the circuit to build the total high-frequency phase shift up to 90°.

The input capacitance of the voltage-recording microelectrode (ME1) adds another frequency-dependent variable into the system which also tends to decrease the stability. The effect of this input capacitance is usually minimized by carefully adjusting the capacitance neutralization control to maximize the bandwidth of ME1.

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 100.)

(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 ME1 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 ME2 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 unless the capacitance neutralization of ME2 is properly set. Under these circumstances the capacitance neutralization of ME2 may have a significant effect on the rise time of the clamp step response since the output capacitance and the microelectrode resistance will effectively form a low-pass filter for the output voltage.

(3) Measures that reduce the stray capacitance of the microelectrodes will also reduce C_x. This would include coating the microelectrodes with Sylgard. **NOTE:** The use of a driven shield on ME1 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 potential differences across this field can be quite 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 is to be less than 5 mV and the current is 10 µA, R_a must be less than 500 ohm. 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 the *cSEVC* section on p. 81).

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.

TROUBLE SHOOTING

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 physically disconnect **all** instruments connected to the Axoclamp-2B except for the oscilloscope. Ideally, remove the Axoclamp-2B from the rack. Work 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.

Questions? Axon's Knowledge Base: http://www.axon.com/mr_Technical_Support.cfm

Voltage Clamp Problems

Some problems and possible causes are listed below.

cSEVC

Symptom: Although the gain can be increased, the speed of the clamp is not sufficient.

Possible cause: The cell capacitance may be too large for the cSEVC mode of the Axoclamp-2B.

Suggestion: Try a lower resistance patch pipette.

Possible cause: The currents are too large to be clamped.

Suggestion: Try the dSEVC mode.

dSEVC

Symptom: The sampling rate cannot be increased to the level appropriate to clamp the cell even after adjusting both the CAPACITANCE NEUTRALIZATION and ANTI-ALIAS FILTER controls.

Possible cause: The microelectrode response may be too slow.

Suggestion: Try a lower resistance microelectrode such as a patch pipette.

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 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 time-course of the voltage step is slower than the current transient. 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.

All Voltage Clamp Modes

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 circuit 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. Observe the V_1 CONT. or V_2 outputs at a wide bandwidth to check that the capacitance neutralization circuit is not in oscillation.

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 ME1 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 *Series Resistance* and, if using TEVC, *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.

Space Clamp

There is one limitation to the performance of the voltage clamp that cannot be electrically compensated. This is the deviation of the cell from a sphere centered on the tip of the voltage-recording microelectrode. The voltage clamp is maintained at the tip of the voltage-recording microelectrode. If all portions of the cell membrane are separated from this tip by equal access resistance, then the membrane will be uniformly voltage clamped. However, many cells have processes such as axons, dendrites and filopodia attached to the cell body (where the microelectrodes are usually located). The membranes of these processes are separated from the cell body by an axial access resistance whose value depends on the distance to each portion of the membrane and the cross section in that region of the cell. Thus there is a voltage drop across the access resistance that becomes substantial for distal components of the membrane. Even though the somatic membrane potential may be well controlled, the axonal or dendritic membrane potential may be very poorly controlled. In these cases, the time course of synaptic currents, regenerative currents and measurements of reversal potentials may be grossly distorted.

As a general rule, the voltage clamp is considered to be acceptable if the length of the attached axon or dendrites is no more than 0.1 length constants. (Even this short length will cause significant distortion of fast currents. See Figure 7 in Rall and Segev, 1985). Calculation of the length constant for a cell is complicated since it depends on the geometry of the particular cell under investigation. Some of the common ways to avoid the problems of poor space clamping are as follows:

(1) Restrict investigations to spherical cells. Many cultured cells are convenient.

(2) Ligate attached axons. For example, the axon of large molluscan neurons can be tied off with nylon thread.

(3) Use short segments. For example, short segments (100 µm) of arteriolar syncytia can be separated from the arteriole by careful cutting with a razor blade.

(4) Restrict the range of the clamp to a short segment of the cell. This is the essence of the "sucrose gap" technique sometimes used on axons.

(5) Restrict the measurement to currents that are generated close to the microelectrodes. For example, the end plate currents in muscle fibers can be well clamped, even though the bulk membrane current is very poorly clamped.

(6) Restrict the measurement to the current flowing through a large patch of membrane, instead of the whole cell. The "macropatch" technique is a special case of the single-channel patch-clamp technique, in which there are sufficient channels for an ensemble current to be recorded.

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.

SPECIFICATIONS

Unless otherwise specified, $T_A = 20^oC$, 1 hr warm-up time.

MODES

Five main operating modes selectable by color-coded illuminated push buttons, or remotely. These are:

1. **BRIDGE**
2. **DCC:** Discontinuous Current Clamp
3. **dSEVC:** Discontinuous Single-Electrode Voltage Clamp
4. **cSEVC:** Continuous Single-Electrode Voltage Clamp
5. **TEVC:** Two-Electrode Voltage Clamp

MICROELECTRODE AMPLIFIERS (Two Channels)

Unity-Gain Headstages: HS-2 and HS-2A are standard. HS-2A are recommended for ME2 to prevent latch up in the high compliance TEVC mode. The "L" type is standard type. The "M" types are the same except: 1) the noise is greater by about 20%; and 2) the capacitance neutralization range is extended. The "MG" types are similar to the "M" types except that the case is grounded instead of driven.

Hum (line-frequency pickup): Less than 10 μV peak-to-peak, grounded input.

Headstage Current Gain (H): Available in 5 values (specify two with order). Select on basis of cell input resistance (R_{in}) and maximum current capacity (I_{max}).

Maximum Currents HS-2 and HS-2A Headstages:

Gain	Maximum Current for ME1*	Maximum Current for ME2* in TEVC Mode	Notes
0.0001MU	0.11 nA	1.30 nA	Driven case. Medium capacitance neutralization range.
0.01MU	11.0 nA	130 nA	Driven case. Medium capacitance neutralization range.
x0.1LU	0.11 μA	1.3 μA	Driven case. Low capacitance neutralization range.
x1LU	1.1 μA	13 μA	Driven case. Low capacitance neutralization range.
x1 MGU	1.1 μA	13 μA	Grounded case. Medium capacitance neutralization range.
x10 MGU	11 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	ME1	ME2	Notes
<300 kΩ to 3 MΩ	HS-2A-x1LU	HS-2A-x10MGU	Oocyte clamping
3 MΩ to 30 MΩ	HS-2A-x1LU	HS-2A-x1MGU	
30 MΩ to 300 MΩ	HS-2A-x0.1LU	HS-2A-x0.1LU	

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

Recommended Headstages for Single-Electrode Voltage Clamping:

Mode	Configuration	Cell Input or Pipette Resistance	ME1	Notes
dSEVC	Whole cell	3 - 30 MΩ	x1LU	Patch pipette
	Whole cell	30 - 300 MΩ	x0.1LU	Intracellular microelectrode
cSEVC	Whole cell	3 - 30 MΩ	x0.1LU	Patch pipette
	Macropatch	1 - 10 GΩ	x0.1LU	Patch pipette
	Macropatch	1 - 10 GΩ	x0.01LU	Patch pipette

Recommendations for Ion-Sensitive Electrodes and Ionophoresis:

Ionophoresis: x1L is suitable for most cases.
Ion-sensitive: x0.0001 electrodes

Noise with Grounded Input: 5 μV rms measured with a 10 kHz single-pole filter in the measurement circuit. Value is for an HS-2A-x1LU headstage.

Noise with a Source Resistance: 51 (47) μV rms measured with a 10 (100) MΩ source resistance and capacitance neutralization adjusted for a 10 (1) kHz bandwidth and with a 10 (1) kHz single-pole-filter in the measurement circuit. Values are for an HS-2-x1LU (x0.1LU) headstage.

1% Settling Time: ME1: 16 (54) μs for a voltage step applied to the input via a 10 (100) MΩ low-capacitance resistor and 16 (60) μs for a current step into the same resistor.

ME2: 40 (110 μs) for a voltage step applied to the input via a 10 (100) MΩ low-capacitance resistor and 40 (120 μs) for a current step into the same resistor.

Capacitance neutralization adjusted for zero overshoot. Values are for an HS-2A-x1LU (x0.1LU) headstage.

Working Input Voltage Range: ±13 V for transients and steady state, protected to ±30 V for ME1. For ME2 in TEVC mode, protected to ±130.

Input Resistance: 10^{14}-10^{15} Ω, H = x 0.0001 (see note)*
10^{13} Ω, H = x .01
10^{12} Ω, H = x 0.1
10^{11} Ω, H = x 1
10^{10} Ω, H = x 10

***Note:** For the x0.0001 headstage, the input resistance of each headstage is measured individually. The unique test results are supplied with each x0.0001 headstage.

Input Capacitance: Not relevant. See 1% settling time and noise specifications.

Input Leakage Current: Adjustable to zero.

Input Leakage Current *vs.* Temperature:

10 fA/°C,	H = x0.0001
100 fA/°C,	H = x0.01, x0.1
1 pA/°C,	H = x1
10 pA/°C,	H = x10

Offset Neutralization Range: ±500 mV. Ten-turn potentiometers.

Capacitance Neutralization Range:

HS-2LU: -1 to 7 pF
HS-2MU: -2 to 20 pF
HS-2MGU: -4 to 18 pF
These values apply when headstage is used with microelectrode 1 amplifier. With microelectrode 2 amplifier the maximum values are doubled.

Buzz: Instantly increases capacitance neutralization to cause oscillation. Operated by spring-loaded push-button switch, footswitch or by Remote Buzz Duration control. The latter allows the Buzz duration to be set in the range 1-50 ms.

Buzz Duration: 1-50 ms when activated by the remote buzz control.

Clear: Forces $\pm I_{max}$ through the microelectrode. Spring-loaded toggle switch.

Bridge Balance Range: 10 ÷ H MΩ/turn in Bridge mode. 1 ÷ H MΩ/turn in cSEVC mode. Ten-turn potentiometers.

Digital Voltmeters:

Voltage Displays: ±1999 mV. Separate meters for V_1 and V_2.

Current Displays:

±19.99 pA,	H = x 0.0001
±1.999 nA,	H = x .01
±19.99 nA,	H = x.0.1
±199.9 nA,	H = x 1
±1.999 mA,	H = x 10

Scaling is set by miniature panel switches. Display selections are I_1, 0.1 x I_2 and I_B.

Currents exceeding the digital display range can be measured on the BNC outputs.

Outputs: 10 V_m and I_m are membrane voltage (gain = 10) and current recorded by microelectrode 1.

V_1 and I_1 are the continuous microelectrode 1 voltage and current.

V_2, 0.1 x I_2 and I_2 are microelectrode 2 voltage and current.

MONITOR is the output of the ANTI-ALIAS FILTER (equals the input of the sampling device). Gain = 10. Baseline correction circuit automatically references Monitor trace to zero volts.

Gain of Current Outputs: 10 ÷ H mV/nA for I_m or I_2 and 1 ÷ H mV/nA for 0.1 x I_2. Maximum output level is ±13V.

Current Outputs indicate the **true** electrode current.

Output Low-Pass Filter Cutoff:

0.1, 0.3, 1, 3, 10, 30 kHz.
Operates on V_m and I_m. Single-pole filter.

Output Impedances: 500 Ω ±10%

VOLTAGE CLAMP

Rise Time (dSEVC): The 10-90% rise time is 100 µs for a 10 mV step command when clamping a 1 nF//10 MΩ model cell via a 10 MΩ microelectrode.

Rise Time (TEVC): The 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 microelectrodes.

Noise (TEVC): Voltage noise (V_1) = 30 µV rms (10 kHz) (150 μV_{p-p}) with MCO-1U model cell (characterized under **OPTIONAL ACCESSORIES** on page 112).

Current noise (I_2) = 140 nA rms (10 kHz) (1 μA_{p-p}) for Gain = 10,000 when clamping the MCO-1U model cell.

Gain: Maximum in dSEVC mode is 100 x H nA/mV.
Maximum in cSEVC mode is 1000 x H nA/mV.
Maximum in TEVC mode is 10,000 mV/mV.
Range is 300:1, logarithmic scale.

Output Compliance: ±25 V in cSEVC and dSEVC; ±130 V in TEVC.

Phase Lag: Multiplier OFF, x0.01, x0,1, x1, x10, x100
Lag range (ms) 0.01-1.0

Anti-Alias Filter: Time constant range 0.2-100 µs

RMP Balance Indicators: Equal brightness indicates voltage clamping will be at resting membrane potential.

Blank: Stops clamp from responding to new inputs for the duration of a HIGH control signal on the BLANK ACTIVATE input. Used to reject stimulus artifacts.

Series Resistance Compensation: Operates in cSEVC mode. Value set on Bridge potentiometer. External input at 100 mV/V can be used in TEVC mode.

SAMPLING CIRCUIT

Rate: 500 Hz to 50 kHz. Operates in DCC and dSEVC modes only.

Counter: 3-digit display to 99.9 kHz max. Blanked in continuous modes.

Sample Clock: Logic-level trigger output at the sampling rate.

Sample Acquisition Time: l μs (10 V step to 0.1%)

INTERNAL COMMANDS

Note: Commands from all sources sum linearly.

Voltage Clamp Step Command: ±199.9 mV. Set on thumbwheel switch. Activated by a HIGH control signal on the STEP ACTIVATE input or by a front-panel switch.

Voltage Clamp Holding Position: Range ±200 mV transmembrane potential. Ten-turn potentiometer.

Current Clamp Step Command: ±199.9 x H nA. Set on thumbwheel as above.

DC Current Command: ±100 x H nA. Ten-turn potentiometers.

EXTERNAL COMMANDS

Sensitivities: Ext. VC command: 20 mV/V
Series resistance compensation: 100 mV/V
Ext. ME 1 (microelectrode 1) command: 10 x H nA/V
Ext. ME 2 (microelectrode 2) command: 10 x H nA/V
Input Impedance: 22 kΩ

Max. Input Voltages: ±30 V for voltage-clamp commands
±60 V for current-clamp commands

CALIBRATION SIGNAL

A pulse equal in magnitude to the setting on the thumbwheel switch is superimposed on the voltage and current outputs for the duration of a HIGH control signal on the CAL ACTIVATE input.

BATH POTENTIAL COMPENSATION

Signal recorded by bath headstage or by an external amplifier is automatically subtracted from the intracellular measurements. If bath potential is not measured the system automatically reverts to using zero volts as the reference potential. Standard headstages (HS-2 or HS-2A) work as bath headstages when plugged into the BATH PROBE (V) connector.

VIRTUAL-GROUND CURRENT MEASUREMENT

A VG-2 virtual-ground headstage can be plugged into the BATH-CLAMP PROBE (I) connector. The current measured is the sum of all currents into the preparation. The correct operation of the Axoclamp-2B is not dependent on the use or of a virtual-ground current measurement headstage.

REMOTE

Logic HIGH control signals activate BUZZ and CLEAR of each microelectrode, and select between BRIDGE, DCC, SEVC and TEVC modes. 15-pin connector.

MODEL CELLS

Two model cells are provided with the Axoclamp-2B. Special plugs connect directly to the headstages.

In the CLAMP-1U model cell electrodes are 50 MΩ and the cell is 50 MΩ//500 pF. A switch grounds the electrodes directly (BATH mode) or through the cell (CELL mode). The ground jack is on the side of the model cell box.

In the MCW-1U model cell electrodes are 10 MΩ and the cell is 500 MΩ//30 pF.

GROUNDING

Signal ground is isolated from the chassis and power ground.

CONTROL INPUTS

Above 3 V is accepted as logic HIGH. Below 2 V is accepted as logic LOW. Inputs are protected to ±15 V.

HEADSTAGE DIMENSIONS

HS-2A and HS-2 case is 2.25" x 1.14" x .0.87" (57.2 mm x 29.0 mm x 22.1 mm). Mounting rod is 4" (102 mm) long. Available mounting rod diameters are 1/4", 5/16" (standard) or 3/8" (6.3 mm, 7.0 mm 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 HOLDER

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.

CASE DIMENSIONS

7" (177 mm) high, 19" (483 mm) wide, 12.5" (317 mm) deep. Mounts in standard 19" rack. Handles are included. Net weight 18 lbs. (8 kg).

SUPPLY REQUIREMENTS

Line voltage: 85 to 264 V_{ac} (110 to 340 V_{DC}) universal voltage input.
Line frequency: 50-60 Hz
Power: 30 W
Fuse: 2.0 A slow. 5 x 20 mm.

ACCESSORIES PROVIDED

Theory and Operation Manual
HS-2A-x1LU headstage
HS-2A-x0.1LU headstage
Two HL-U electrode holders.
Other HS-2A or HS-2 headstages may be substituted on request with order.
2 mm plugs for use with headstages
Low-capacitance test resistor for each headstage.
Spare globes for Mode switches
Spare fuse
Footswitches to operate Buzz of both electrodes
CLAMP-1U & MCW-1U model cells
Remote Buzz Duration hand-held control

OPTIONAL ACCESSORIES

(ordered separately at additional cost; not required for normal operation)

HS-4-x1MGU Relay-Switched Headstage.

Miniature relay inside headstage automatically bypasses the current-measuring resistor during two-electrode voltage clamp mode. In all other modes the HS-4 series headstage behaves like an HS-2A-x1MGU headstage. Must be used in conjunction with a VG-2 virtual-ground headstage.

VG-2 and VG-2A virtual-ground headstages.

Measure the total bath current. The virtual-ground output attenuation (VG) is available in four values (specify with order): x0.1, x1 , x10 and the VG-2A-x100. The output (I_{BATH}) is 10 ÷ VG mV/nA. Clamps the bath potential to zero volts. The VG-2A-x100 is recommended when voltage clamping oocytes.

MCO-1U model cell

Models an oocyte, bath current and sense electrodes and two microelectrodes. A sealed membrane patch is modeled as well.

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

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STANDARD WARRANTY AND REPAIR SERVICE

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.

ADVISORY REGARDING SHIPPING

Shipping the Axoclamp-2B

The Axoclamp-2B is a solidly built instrument designed to survive shipping around the world. However, in order to avoid damage during shipping, the Axoclamp-2B must be properly packaged.

In general, the best way to package the Axoclamp-2B is in the original factory carton. If this is no longer available, we recommend that you carefully wrap the Axoclamp-2B in at least three inches (75 mm) of foam or "bubble-pack" sheeting. The wrapped instrument 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 Axoclamp-2B. If the carton is dropped by the shipper, there is a good chance that the instrument will shift within the loose pellet packing and be damaged.

If you need to ship the Axoclamp-2B 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 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 the packaging is inadequate, and the instrument is damaged during shipping, the shipper will not honor your claim for compensation.

DECLARATION OF CONFORMITY

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

Type of Equipment: Scientific Instrument (Picoammater)

Model Number: AxoClamp-2B

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)*

CE

CIRCUIT DIAGRAMS REQUEST FORM

All the information that you require for operation of the Axoclamp-2B is included in the operator's manual. In the normal course of events, the Axoclamp-2B does not require any routine maintenance.

Should you need the circuit diagrams for the Axoclamp-2B, Axon Instruments will be pleased to supply them to you. However, we caution you that the Axoclamp-2B 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:

Axon Instruments, Inc.
Sales Department
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: Axoclamp-2B Serial number: _____

Declaration
Please send me the circuit diagrams and parts lists for the Axoclamp-2B. I agree that I will only use the circuit diagrams and parts lists for service of the Axoclamp-2B. 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 Axoclamp-2B, I will ask them to make the same undertaking that I am declaring herein.

Signature: _____ Date: _____

Name: _____ Title: _____

GLOSSARY

C_{in}	Total input capacitance of the headstage due mainly to the microelectrode and any connecting cable
C_m	Membrane capacitance of cell
cSEVC	Continuous single-electrode voltage clamp
DCC	Discontinuous current clamp
dSEVC	Discontinuous single-electrode voltage clamp
f_s	Sampling rate; rate for switching from current passing to voltage recording in DCC and dSEVC modes
G	The average gain during dSEVC
G_T	The instantaneous gain of the controlled current source during dSEVC
H	Headstage current gain
I_1, I_2	Continuous current flow in microelectrode 1, Current flow in microelectrode 2
0.1 x I_2	Current flow in microelectrode 2 attenuated by ten
I_m	Membrane current flow
Lag	High-frequency cut
ME1, ME2	Microelectrode 1, Microelectrode 2
R_b	Bath electrode resistance
R_e	Intracellular electrode resistance
R_s	Resistance in series with membrane
RMP	Resting membrane potential
R_m, R_{in}	Input resistance of cell membrane
SEVC	Single-electrode voltage clamp
TEVC	Two-electrode voltage clamp
V_1 CONT.	Continuous voltage recorded by microelectrode 1
V_2	Voltage recorded by microelectrode 2
VC	Voltage Clamp
VG	Virtual-ground output attenuation
V_m	Membrane potential recorded by microelectrode 1
μ	Gain of voltage clamp

Aliasing, 59
definition, 59
Anti-alias filter, 21, 24, 55, 59
to reduce aliasing, 59
use in cSEVC, 18
use in TEVC, 59, 94

Bath error potential (Vb), 59
clamping Vb, 61
compensating series resistance, 62
measuring and subtracting Vb, 61
methods of minimizing, 60
Blanking, 45
use of, 45
Bridge mode, 6, 81
theory of operation, 81
tutorial, 13
Buzz, 45
remote control of, 46
uses of, 45

Calibration signal, 46
step input, 46
Capacitance neutralization, 46, 64
adjustment of, 21, 64
input capacitance, 64
methods of, 64
Chlorided Wire, 51
Clear, 47
remote control of, 47
uses of, 47
Command generators, 47
DC command, 48
external command, 48
mixing commands, 48
step command, 47
Computer control, 39
interfacing with, 39
remote control and sequencing, 39
Continuous single-electrode voltage clamp (cSEVC), 17, 81
comparison to dSEVC, 91
series resistance, 18, 78, 83
theory of operation, 81
whole-cell clamp, 84
Current conventions, 66
Current measurement, 65
headstage gain, 65
I display meter, 65
outputs for, 65

Discontinuous current clamp (DCC) mode, 85
comparison with Bridge, 85
noise, 93
theory of operation, 85
tutorial, 20
Discontinuous single-electrode voltage clamp (dSEVC)
appropriate headstages, 87
clamp error, 90
comparison to cSEVC, 91
false clamp, 25
gain, 90
maximum gain, 89
minimum sample rate, 21, 89
noise, 91, 93
series resistance, 78, 90
tutorial, 23
Filtering
external filter, 68

Glass
Dimensions, 53
Grounding and hum, 40

Headstages, 69
cleaning, 74
connectors, 73
DC removal, 76
gain (H), meaning of, 69
HS-2 compatibility, 69
HS-2 selection of, 70
HS-2A, 49
HS-4, 50, 72
input leakage current, 74
input resistance, 76
interchangeability, 74
types of, 49
virtual ground, 50
bath clamp, 50
Holder
Pipette, 51
Holder, Pipette
Cleaning, 52
Glass Dimensions, 53
Use, 52
Holding position
use of, 58

Ionophoresis, 54
setting current commands for, 54
Link-up, 54
use of in dSEVC, 54

Macropatch, 67
IN CSEVC MODE, 85
Microelectrodes, 31
capacitance, 31
fast settling time, 31
filling solutions, 34
glass, 33
patch pipettes, 36, 90
glass, 36
seal formation, 37
settling time, 34
stability, 34
tip potentials -- detection, 33
tip potentials -- prevention, 34
tip resistance, 34
types of, 33
Micropipettes, 33
cell impalement, 35
filling solutions, 34
glass, 33
settling time, 34
stability, 34
tip potentials -- detection, 33
tip potentials -- prevention, 34
tip resistance, 34

Model cells, 41
making of, 43
types of, 41

Noise, 104

Offset controls, 76
input offset, 76
output offset, 76
Output filter, 77
low noise recording, 77
risetime, 77
Output impedance and protection, 55
Outputs
current outputs (Im), 80
monitor, 55
voltage outputs
10 Vm, 80

Panel meters, 56
current display (0.1 x I2), 56
current display (Ibath), 56
current display (Im), 56
voltage display (V2), 56
voltage display (Vm), 56
Phase lag, 25, 78
use of, 30, 78
Potentiometers
ten-turn, 44
Power supply, 41
fuse changing, 41, 56
voltage, 56
Power-off loading, 39

Remote control, 56, 58
buzz, clear and mode selection, 46, 47, 56
hand-held buzz remote, 46

Sample rate, 24
rate adjust knob, 21
Series resistance, 78
definition of, 78
sources of, 78, 83
Shielding, 65, 73
driven shield, 65, 73
in TEVC, 74
Space clamp, 103

Trouble shooting, 101
Two-electrode voltage clamp (TEVC), 94
appropriate headstages, 96
comparison to SEVC, 95
coupling capacitance
shielding, 99
intracellular electrode coupling, 100
series resistance, 78, 95
stability of, 98
tuning, 28, 98
tutorial, 27
Virtual ground current measurement, 79
Voltage clamp
problems, 101
Voltage conventions, 66
Warnings, 39, 73, 75, 97
HS-4 headstages, 73
power-off loading, 39
TEVC mode, 97