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CARACTERISTICAS ANATOMO-FISIOLOGICAS DE NERVIOS Y MUSCULOS DEL AREA PELVICA

TESIS

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ABSTRACT

In mammals, the regulatory mechanism exerted by the lumbrosacrococcigea of the spinal cord on pelvic function are still largely unknown. This is due not only to the anatomical complexity of the various muscles and viscera, but also to the fine and intricate neural net which innervates them.

It was the aim of this thesis to analyse and describe in mammals the interaction of the various muscles and nerves of the pelvic area which contribute to the reflexes underlying the expression of reproductive function, retention and expulsion, and chemical communication. The results are divided into six studies representing an integrative approach in which pelvic processes are considered from an anatomical, physiological and behavioral point of view.

The first study analysis the effect of genital stimulation on spinal reflexes in spinal cats under various hormonal conditions. The second study demonstrates how in spinal cats the neural apparatus of the striated muscles is capable of differentially activating the muscles. In the third study the efferent innervation by the pelvic and pudendal nerves is characterized in female rats. The fourth study, also in female rats, describes various muscular reflexes produced by genital stimulation, the nerves involved, and the visceral and postural effects induced by these reflexes. In the fifth study the role of the branches of the pelvic nerve in mediating copulatory behavior in the male rat is investigated. The final study describe the anatomical position of the striated muscles of the pelvic area in relation to the reproductive tract in the female rabbit.

Together, the results provide valuable information, from an anatomical and functional point of view, permitting a more integrated understanding of the diverse functions associated with the pelvic area in mammals.

RESUMEN

Los mecanismos de la regulación que ejerce la médula espinal lumbosacrococcígea sobre las funciones del área pélvica en mamíferos aún se desconocen en una gran parte. Ello se debe probablemente no solo a la complejidad anatómica de las diferentes vísceras y músculos, sino también a la fina e intrincada red neural que inerva tales estructuras.

La presente tesis tuvo por objeto el análisis y descripción en mamíferos de las interacciones de diversos músculos y nervios del área pélvica que forman parte de reflejos que permiten la expresión de funciones reproductivas, de expulsión-contención y de comunicación química. Los resultados se presentan divididos en seis trabajos que representan un enfoque integrativo en los que los procesos pélvicos son considerados desde un punto de vista anatómico, fisiológico y conductual.

El primer trabajo analizó los efectos de la estimulación genital sobre los reflejos espinales de gatas espinalizadas bajo varias condiciones hormonales. En el segundo trabajo se demostró en gatos espinalizados que el aparato neural de la musculatura estriada pélvica es capaz de activar diferencialmente a los músculos. En el tercer trabajo se caracterizó en ratas hembras la inervación eferente de los nervios pélvico y pudendo. En el cuarto trabajo se describieron también en rata hembra, varios reflejos musculares producidos por estimulación genital, los nervios que participan y los efectos viscerales y posturales inducidos por dichos reflejos. En el quinto trabajo se investigó el papel de las ramas del nervio pélvico en la mediación de la conducta copulatoria de la rata macho. En el último trabajo se describió en coneja la disposición anatómica de los músculos estriados del área pélvica en relación al tracto reproductivo.

Los resultados en su conjunto aportan información valiosa desde el punto de vista anatómico y funcional que permite un entendimiento más integral de las diversas funciones que tienen como sustrato el área pélvica de los mamíferos.

INTRODUCCION

A nivel fisiológico, la conducta sexual en mamíferos representa la interacción entre redes neuronales y endocrinas del macho y la hembra. Durante el cortejo, la emisión de señales odoríferas provenientes del área genital, puede ser determinante para arribar a la cópula. Ya en el apareamiento, diversas partes del cuerpo se deben acoplar con precisión: algunas de estas áreas están localizadas en su sistema urogenital o, de manera más general, en el área pélvica.

En la región pélvica de los mamíferos se observa una gran complejidad anatómica y funcional. Esta complejidad se acrecienta en primates por su posición erecta, lo cual promovió una serie de cambios en la disposición de sus vísceras y en su musculatura pélvica (Wilson, 1973b).

Las vísceras pélvicas y los músculos que las rodean forman una unidad funcional de considerable complejidad, cuya eficiencia depende de muchos factores. De éstos, quizá el más importante sea la extensa y rica inervación de esta región, que le permite una gran sensibilidad y una actividad incesante.

La actividad refleja desencadenada en el área pélvica de la hembra por la estimulación genital, es solo una parte de las respuestas producidas por el apareamiento.

La lordosis involucra elevación de la cabeza, dorsiflexión de la espalda (depresión del tórax, elevación de la cadera y de la base de la cola), desviación lateral de la cola y extensión bilateral de las patas posteriores (Pfaff y Lewis, 1974). Varios músculos posturales dan cuenta del mantenimiento de la extensión de las patas. Uno de ellos es el músculo gastrocnemio.

En mi laboratorio se hizo un estudio en gatas espinalizadas sobre los efectos que producía la estimulación genital sobre la actividad refleja de la médula espinal espinal lumbar (*primer trabajo* de esta tesis; Pacheco y col., 1976). La estrategia utilizada fue estimular eléctricamente los nervios sural y gastrocnemio (cutáneo y muscular, respectivamente) y registrar la actividad eléctrica de las raíces ventrales L7 y S1 antes, durante y después de la estimulación genital (golpeteo de la piel perivulvar y presión ligera en el tracto vaginal). Se encontró que la estimulación del nervio sural produjo a nivel de raíz ventral, una respuesta con dos componentes polisinápticos, y que la estimulación del músculo gastrocnemio producía la respuesta con un componente monosináptico seguida de un componente polisináptico. La estimulación táctil del área cutánea perivulvar o la de la nuca, inhibió la respuesta polisináptica del sural y del gastrocnemio y facilitó la respuesta monosináptica por la estimulación del gastrocnemio. La estimulación vaginal y cervical provocó que en la mayoría de los casos, el reflejo monosináptico se deprimiera y además que se inhibiera la respuesta polisináptica por la estimulación del gastrocnemio. Se observó aumento de la respuesta monosináptica por la estimulación del gastrocnemio cuando se suspendió la estimulación cervical. En experimentos adicionales se observó que la sección bilateral de los nervios pudendos, aunque disminuyó, no bloqueó completamente los efectos obtenidos por el golpeteo de la piel genital. Los efectos obtenidos fueron independientes de la condición endócrina de los animales.

Estos hallazgos resultaron ser una excepción a la regla Sherringtoniana, esto es, se encontró la facilitación de motoneuronas de un músculo extensor por la estimulación de un nervio cutáneo; también se observó que la misma estimulación cutánea causaba inhibición de la actividad polisináptica producida por estimulación del nervio sural, en lugar del incremento previsto. El primer dato apoyó la idea de una facilitación de la actividad muscular extensora durante la lordosis por la estimulación vaginocervical (Komisaruk y Diakow, 1973), y el segundo grupo de datos dio apoyo a la proposición de que la estimulación vagino-cervical provoca analgesia (Komisaruk y Larsson, 1971).

Las vísceras del área pélvica incluyen a estructuras como la vagina, el cérvix y el útero, que participan en las funciones sexuales, y a estructuras de expulsión y de contención como el recto y la vejiga. Las analogías entre todas ellas son su función de reservorios, la presencia de ángulos y curvaturas, los arreglos para la sujeción y apoyo entre ellas, y los dispositivos de sellado que facilitan sus continencias (Wilson, 1973a).

La sujeción y apoyo de las vísceras pélvicas es realizado por la compleja organización de músculos y fascias. Elftman, en su análisis sobre la evolución del piso pélvico en primates destaca que "la característica sobresaliente es su pérdida de desarrollo muscular, y que puede resistir el esfuerzo continuo sin gasto de energía, pero no puede proporcionar poder motor" (citado por Wilson, 1973b).

Los músculos del piso pélvico desarrollan uniones y funciones específicas. Sus acciones sobre el movimiento de la cola en algunas especies, son secundarias a las de la verdadera musculatura caudal que se desarrolla independientemente (Wilson, 1973b).

Los arreglos musculares son fundamentales a la cooperación entre las vísceras y sus alrededores. Así mismo lo es el que los segmentos espinales lumbares y sacros, que inervan las vísceras pélvicas, también inerven a los músculos circundantes y a la piel supraadyacente. Como resultado, se han desarrollado circuitos neurales de retroalimentación de gran sutileza; ellos controlan las funciones con mucha precisión y ofrecen un reto al entendimiento humano.

En el hombre se reconoce la importancia de los músculos del diafragma pélvico para la ejecución precisa de las funciones de contención, expulsión y reproductivas (p. ej. Kegel, 1952; Porter, 1962; Wilson, 1973b; Wendell-Smith y Wilson, 1977; Gillan y Brindley, 1979; Graber, 1982; Koelbl y col., 1989; Dubrovsky y Filipini, 1990), pero aún queda mucho por conocer sobre su fisiología.

Una noción que prevalecía en la literatura, era que los músculos del piso pélvico se comportan como una masa unitaria (Porter, 1962; Wendell-Smith y Wilson, 1977; Dickinson, 1978). Esta proposición era justificada pues en el hombre no se distinguen claramente los límites musculares, pero era difícil de aceptar si consideramos que estos músculos, además de su función secundaria como sostén para las vísceras pélvicas, participan en funciones tales como la continencia, la defecación y la micción, y también contribuyen a que las funciones reproductivas y sexuales se manifiesten, todo lo cual parece indicar un ajuste neural muy fino y preciso que no encaja con una visión de actividad contráctil "en masa".

En nuestro laboratorio se analizó esta proposición experimentalmente (*segundo trabajo* de esta tesis; Dubrovsky y Pacheco, 1980; Dubrovsky y Pacheco, 1981; Dubrovsky y col., 1985). En gatos con sección espinal alta, registramos actividad electromiográfica (EMG) de los músculos esfínter externo del ano, abductor cauda interno, levator ani y sacrocaudal dorsal lateral. El segundo y tercer músculo forman el diafragma pélvico que cierra la cavidad pélvica del gato (Crouch, 1969). La estimulación eléctrica de las raíces ventrales S2 produjo contracción del esfínter externo del ano y del sacrocaudal dorsal lateral. La estimulación eléctrica de las raíces ventrales S3 y Co1, produjo respuestas en el levator ani y el abductor cauda interno. Así se demostró una clara separación de las poblaciones de motoneuronas que inervan los diferentes músculos del piso pélvico. Estos músculos del piso pélvico pueden ser activados reflejamente, de manera individual o como una masa unitaria dependiendo de la intensidad del estímulo. La estimulación táctil o eléctrica de las regiones pudendas, de cualquier lado del cuerpo, produce respuestas en el esfínter externo del ano. En cambio, la activación del levator ani y del abductor cauda interno pudo ser lateralizada: la estimulación eléctrica o táctil de las superficies dorsolaterales de la base de la cola produjo respuestas ipsilaterales de estos músculos. La sección unilateral del nervio pudendo no alteró el nivel de actividad tónica (2-4/seg) del esfínter externo del ano. Sin embargo, la sección bilateral del nervio pudendo si acabó con su actividad tónica y con la responsividad fásica de este músculo, pero no afectó la actividad del levator ani ni la del abductor cauda interno. La estimulación del nervio pudendo produjo solamente actividad refleja polisináptica en las raíces ventrales S2.

Nuestros hallazgos de que el esfínter externo del ano responde a la estimulación de cualquier lado del cuerpo, está de acuerdo con los resultados de otros autores (Bishop, 1959; Gunterberg y col., 1976; Jolesz y col.,1982; Wunderlich y Swash, 1983), quienes también confirmaron las observaciones de Sherrington en 1892 de la superposición en la distribución de fibras motoras de los nervios pudendos derecho e izquierdo. Los hallazgos de que la actividad de los músculos compresores de la cloaca (diafragma pélvico) puede lateralizarse, coinciden con los resultados que fueron obtenidos durante intervenciones quirúrgicas realizadas por Percy y col. (1980), en un estudio sobre la inervación motora de los músculos del piso pélvico en el hombre, en el cual encontraron una lateralización de la inervación del músculo puborectalis.

Los nervios pudendos inervan los músculos estriados de los esfínteres del ano y de la uretra y a la piel de la región perigenital (Reichard y Jennings, 1963; Bradley y Teague, 1972; Crouch, 1969). Los esfínteres anal y uretral poseen músculos con husos musculares (Chennels y col., 1960; Todd, 1964; Oliver y col., 1970; Shuster, 1975). Sin embargo, en nuestro estudio no pudimos obtener respuestas monosinápticas cuando registramos la actividad refleja en las raíces ventrales sacras, durante la estimulación eléctrica de los nervios pudendos.

Jankowska y col. (1978) y Mackel (1979), encontraron que los PEPSs producidos en motoneuronas esfintéricas por la estimulación de la raíz dorsal, tenían amplitudes menores a 1.0 mV, mientras que aquellas provocadas por la estimulación del nervio pudendo, raramente excedían 0.5 mV. No solamente estas conexiones sinápticas eran débiles, sino que también eran pocas en número; menos de la mitad de las motoneuronas esfintéricas probadas exhibieron PEPSs monosinápticos. Estos hallazgos, poca amplitud de PEPSs y baja frecuencia de conectividad, podrían explicar la ausencia de respuesta monosináptica en raíz ventral sacra, durante la estimulación del nervio pudendo. Nuestra información fue confirmada por Jolesz y col. (1982).

Estos resultados mostraron que el aparato neural de la musculatura estriada del piso pélvico, es capaz de activar individualmente los diferentes músculos que lo forman, y que el esfínter externo del ano, por una parte, y los músculos que conforman el diafragma pélvico por la otra, están controlados por diferentes circuitos neuronales.

En gato y en perro se considera que el diafragma pélvico o levator ani está formado por los músculos iliococcígeo, pubococcígeo y coccígeo (Bradley y Grahame, 1943; Crouch, 1969). Por otra parte, en ratas existió una notable confusión anatómica. Un músculo fue identificado como el "levator ani" en la anatomía de rata de Greene (1935), y este mismo nombre persistió, especialmente en la literatura endocrina. Se anotaba que sólo existía en el macho. Más tarde se encontró que correspondía al músculo bulbocavernoso dorsal (Hayes, 1965), y aunque hubo intentos de reiniciar la controversia (Cihak y col., 1970), se ha corroborado y aceptado la propuesta de Hayes (McKenna y Nadelhaft, 1986).

En la rata, es ahora posible la distinción de los componentes del piso pélvico. Existen algunos estudios en macho sobre las motoneuronas que los inervan (Schroder, 1980; McKenna y Nadelhaft, 1986) y sobre su participación en los procesos de micción (Manzo y col., 1991); en la hembra, una descripción anatómica ha sido hecha por Brink y Pfaff (1980), quienes revisaron algunos de los músculos vertebrales involucrados en la lordosis.

Se considera que los músculos iliococcígeo, pubococcígeo y coccígeo son músculos vertebrales sacrocaudales (Brink y Pfaff, 1980) y pertenecen al grupo compresor de la cloaca (Wendell-Smith y Wilson, 1977). Surgen de la cara medial del hueso innominado de la pelvis y se insertan sobre las vértebras caudales proximales. En la rata albina, los músculos pubococcígeo y coccígeo, son difíciles de separar uno de otro (Brink y Pfaff, 1980). Estos dos músculos algunas veces han sido descritos bajo un único nombre, abductor cauda externo (Greene, 1935). Sin embargo, Rinker en 1954, describió la inervación separada de los dos músculos (citado por Brink y Pfaff, 1980). Se cree que los músculos pubococcígeo y coccígeo corresponden a las porciones anterior y posterior, respectivamente, del levator ani del hombre.

Músculo Iliococcígeo (iliocaudalis, abductor cauda interno): las fibras del músculo iliococcígeo tienen un origen carnoso en el borde ventromedial de la cresta ilíaca desde la muesca glútea (muesca ciática mayor) a nivel del acetábulo. Las fibras pasan caudalmente y desarrollan dos tendones que se continuan con el músculo trasverso caudal, corriendo inicialmente a lo largo del borde lateral de la cola ventral. Conforme los tendones salen de la pelvis, cruzan sobre la línea media de la cola ventral, y se continuan para correr caudalmente. Los tendones se insertan ipsilateralmente sobre las protuberancias ventrales (localizadas medialmente) de Ca5 y Ca6.

Músculos Coccígeo y Pubococcígeo (abductor cauda externo): las fibras de los músculos pubococcígeo y coccígeo forman juntas una vaina muscular delgada, aplanada y triangular que se extiende desde la superficie ventromedial del pubis, para insertarse a lo largo de la diapófisis de la cola proximal. Las inserciones caudales están sobre las fascias de la superficie ventrolateral de la cola, a nivel de Ca6. Los origenes de las fibras son carnosos; las inserciones son tanto carnosas como por medio de fascias de fibras cortas .Los dos músculos son fácilmente separables anteriormente, donde dos vainas de fibras pueden distinguirse de acuerdo al origen de las fibras y a la dirección de la fibra transversal. La vaina lateral (que descansa justo medial al hueso innominado) está formada de las fibras del músculo coccígeo. Estas fibras surgen de la cara medial del hueso innominado, anterior al forámen obturador y a lo largo de su borde ventral medial. Las fibras pasan dorsocaudales para insertarse, al menos, sobre las diapófisis de Ca1 a Ca3. Las fibras del músculo coccígeo hacen las inserciones anteriores viajando casi directamente dorsales. Las fibras del músculo pubococcígeo, que comprenden la vaina medial, viajan más directamente caudales. Estas fibras surgen del borde ventromedial del pubis al nivel de y posterior a la eminencia iliopectineal, y se insertan al nivel de Ca3 y Ca4. Los tendones del músculo iliococcígeo pasan mediales al músculo pubococcígeo. Unas pocas fibras de origen del pubococcígeo pueden unirse al tendón del iliococcígeo.

En el *tercer trabajo* de esta tesis (Pacheco y col., 1989) se caracterizó la inervación eferente de los nervios pélvico y pudendo identificando los músculos activados por estimulación eléctrica en un punto distal a donde ellos se bifurcaron del tronco L6-S1. La estimulación eléctrica del nervio pélvico produjo contracción monitoreada por EMG de los músculos ipsilaterales iliococcígeo y pubococcígeo, que fue bloqueada cuando se cortó una rama (la muscular) del nervio bifurcado. Esta rama recibe entrada propioceptiva activada por el desplazamiento de la cola, mientras que la otra rama, la viscerocutánea, recibe inervación sensorial de la región perineal de la línea media. La estimulación eléctrica del nervio pudendo produjo contracción de los músculos coccígeo, esfínter externo del ano e isquiocavernoso. Movimientos del orificio y pared vaginal fueron directamente visualizados durante la estimulación eléctrica de los dos nervios. La presión intravaginal medida por un globo aumentó por la estimulación eléctrica del nervio pélvico y disminuyó por la estimulación del nervio pudendo. La contracción refleja de los músculos iliococcígeo y pubococcígeo fue producida por emcanoestimulación del periné, vaina clitorial y vagina distal. Esta respuesta fue bloqueada por la estimulación cervical suave.

Una implicación de este hallazgo es que el paso del feto a través del cérvix durante el parto puede relajar los músculos iliococcígeo y pubococcígeo, facilitando con ello el alumbramiento.

Pfaff ha propuesto que en la rata en lordosis los músculos responsables de la dorsiflexión de la espalda son el longísimus lateral y el transverso espinal lumbar, y los responsables de la desviación lateral de la cola, el longísimus medial, el extensor cauda medial y el abductor cauda medial (Brink y col., 1980; Pfaff y Schwartz-Giblin, 1988). Existen sin embargo, otros músculos que no solo se activan durante la postura lordótica, sino que probablemente también contribuyen al mantenimiento de ella. Así por ejemplo, en la rata hembra, los músculos abdominales se observan contraídos por la estimulación vaginocervical durante la cópula.

Durante el parto, estos músculos se activan también por la estimulación vaginal. En un estudio de Higuchi y col. (1987) realizado en ratas periparturientas anestesiadas con uretano, se mostró que la distensión vaginal producía contracciones de músculos abdominales y del diafragma, y que éstas llevaban a un aumento de la presión intra-abdominal. Higuchi y col. llaman a esta contracción muscular refleja el "reflejo de expulsión del feto", y sugieren que inicia los movimientos de pujar durante el parto. Compararon este reflejo en ratas embarazadas normales y en ratas embarazadas con sección de los nervios pélvicos. Se había descrito que el parto es bloqueado en ratas con sección bilateral del nervio pélvico, atribuyéndose su causa al bloqueo de la activación lútea provista por la estimulación del cérvix (Kollar, 1953; Carlson y De Feo, 1965; Spies y Niswender, 1971). Sin embargo, ahora se sabe que las ratas neurectomizadas del pélvico son capaces de un embarazo normal si el cuerpo lúteo es activado de otra manera, aunque el alumbramiento no se lleva a cabo (Carlson y De Feo, 1965). La naturaleza de este bloqueo no había sido investigado con más detalle. Ya que la sección unilateral del nervio pélvico no tiene efecto sobre la expulsión (Burden y col., 1980), se asumió que los nervios pélvicos controlan el proceso del parto por un efecto sistémico de "todo o nada". Higuchi y col. (1987) indican que la principal anormalidad en el parto de ratas con sección del nervio pélvico, es su prolongación, y que ésta puede ser debida a una falta del reflejo de expulsión del feto, que juega el papel fisiológico de mover el feto desde la porción cranial de la cavidad vaginal, en contra de la resistencia opuesta por la salida pélvica.

Los músculos abdominales son divididos en dos grupos, los ventrolaterales y los posteriores. En el segundo grupo quedan comprendidos los músculos psoas mayor, psoas menor, ilíaco y cuadratus lumbar (Greene, 1935; Hebel y Stromberg, 1976). En el hombre y otros primates, el psoas mayor, el psoas menor y el ilíaco, se agrupan juntos como el músculo iliopsoas, y se describe que su contracción flexiona el muslo y el tronco (Wilson y Wilson, 1978). En rata, el origen del psoas mayor está sobre los cuerpos y la superficie ventral de las diapófisis transversas de las vértebras L2 a L6; el origen del músculo ilíaco está sobre las diapófisis transversas de L5 y L6 según Greene (1935), y Hebel y Stromberg (1976) indican que también se origina de la superficie ventral del hueso ilíaco. La inserción de ambos músculos está sobre el trocánter menor del fémur a través de un largo tendón.

Estos músculos, si se asume que contribuyen también a la expresión de la postura lordótica, podrían ser modificados de alguna manera, por la estimulación perigenital y vaginocervical.

En el *cuarto trabajo* incluido en esta tesis (Martínez-Gómez y col., 1992), se describen varios reflejos musculares producidos por estimulación genital, los nervios que los median y los efectos posturales y viscerales inducidos por estos reflejos. La estimulación eléctrica de los músculos iliococcígeo y pubococcígeo produjo movimiento del orificio y pared vaginal, uretra membranosa, cola y pelvis. La estimulación eléctrica de los músculos estriados psoas mayor e iliacus produjo movimientos de las vertebras lumbares y extensión de las patas traseras ipsilaterales. La estimulación de la piel perineal, vaina clitorial o vagina distal, produjo contracción refleja de los músculos iliococcígeo y pubococcígeo. La estimulación del cérvix produjo contracción refleja de los músculos psoas mayor e ilíaco y también bloqueo la contracción refleja de los músculos iliococcígeo y pobococcígeo arriba descrita. Tanto el bloqueo inducido por la estimualción cervical de la respusta refleja del iliococcígeo y del pubococcígeo, como la activación inducida por la estimulación cervical de los músculos psoas mayor e ilíaco, fue evitada por la transección bilateral de la rama viscerocutánea del nervio pélvico. Basados en estas observaciones, se propuso que la estimulación de la superficie vaginal del cérvix resultante de la estimulación peneana y /o de la deposición del tapón seminal durante la conducta de apareamiento en la rata, puede activar reflejamente los músculos psoas mayor e ilíaco, contribuyendo así a la rígidez postural de los miembros traseros y a la dorsiflexión lordótica que son características en la postura de apareamiento en las ratas hembras.

Siguiendo este mismo razonamiento, estuve interesado por conocer si en machos se observaba la misma distinción de los componentes del nervio pélvico y si estaban implicados por ello los músculos iliococcígeo y pubococcígeo (*quinto trabajo* de esta tesis; Lucio y col., 1994). Además, se investigó el papel de estas ramas en la mediación de la conducta copulatoria. La rama somatomotora o viscerocutánea del nervio pélvico fue bilateralmente seccionada en ratas machos con experiencia sexual. La cirugía de la rama somatomotora no tuvo un efecto detectable. La sección de la rama viscerocutánea alteró los parámetros copulatorios que reflejan alteraciones en la erección penil y la emisión del tapón seminal. Los parámetros conductuales alterados alcanzaron los valores de los animales testigos y prequirúrgicos, indicando que el daño a la función eréctil y eyaculatoria fue pasajera. Se sugirió que los animales con sección de la rama viscerocutánea recobran la eficiencia copulatoria a través de un mecanismo plástico compensatorio que posiblemente involucra al nervio hipogástrico.

La abundante actividad refleja postural y visceral implicada en el coito, es integrada en primer lugar, a nivel de la médula espinal.

La parte terminal de la médula espinal, incluyendo los últimos segmentos lumbares, los sacros y los coccígeos, dá origen, en la mayoría de los mamíferos cuadrúpedos, a la inervación sensorial y motora de las regiones pélvica, pudenda y caudal. Es así, uno de los componentes fundamentales de la actividad refleja de estas áreas y donde una gran complejidad anatómica y funcional se observa. En ella convergen los reflejos de varios tipos: somatosomáticos, víscero-viscerales, víscero-somáticos, somato-viscerales, víscero-autonómicos y autonómico-viscerales (Wilson, 1973a; Jänig, 1985; Jänig y McLachlan, 1987).

Las características estructurales y funcionales de esta región fueron observadas en gato por Lloyd y Wilson (1959): "en la parte caudal de la médula espinal, específicamente los últimos segmentos sacros y caudales, el control reflejo está relacionado con las prominentes estructuras de la línea media. Los segmentos terminales de la médula espinal se diferencian de los ensanchamientos, distinguiéndose por la presencia de un gran núcleo dorsal de la línea media, por el crecimiento de la comisura gris dorsal y por la gran decusación de fibras aferentes primarias". Estas características fueron confirmadas por estudios posteriores (Sprague y Ha, 1964; Rethelyi y col., 1979).

En la médula espinal sacrococcígea de gato y mono, Rethelyi y col., (1979), mostraron una proyección abundante de las fibras aferentes primarias a la zona marginal (lámina I), a la sustancia gelatinosa de Rolando (lámina II), y al núcleo propio (láminas III y IV). Las proyecciones a estas tres áreas mostraron ser sustancialmente independientes. Existe también una importante proyección cruzada de fibras aferentes primarias que pasa por la comisura dorsal a la zona marginal y al núcleo propio (Rethelyi y col., 1979; Matsuchita y Tanami, 1983), y que contribuye a la expansión de la comisura dorsal característica de los últimos segmentos espinales lumbares y sacros.

En rata, las fibras aferentes primarias siguen el mismo principio de organización de otros mamíferos, en lo que respecta a su distribución en el asta dorsal: las fibras más delgadas terminan en las láminas superficiales, y las fibras más gruesas llegan a las láminas más profundas (Light y Pearl, 1979). Sin embargo, fibras aferentes primarias viscerales, presumiblemente finas, terminan en las láminas VII y X (Grant, 1985).

En el borde laterodorsal de la lámina VII de los segmentos L6 y S1, está la zona de traslape entre los núcleos simpáticos y parasimpáticos (Nadelhaft y Booth, 1984). El núcleo parasimpático sacro del borde dorsomedial de la lámina VII de L6 y S1, recibe conexiones primarias directas de fibras aferentes primarias finas (Grant, 1985).

El patrón dendrítico exhibido por estas neuronas preganglionares en rata, es similar al patrón correspondiente en gato (Nadelhaft y Booth, 1984). Las dendritas reciben influencias descendentes de los centros de micción del tallo cerebral (Kuru, 1965), así como de las aferentes viscerales pélvicas y de las provenientes de estructuras somáticas importantes para las funciones de micción, defecación y copulación (De Groat y col., 1981).

Las motoneuronas de la médula espinal lumbosacra están distribuidas en varios núcleos, y no se puede hablar de una verdadera lámina IX en rata (Molander y col., 1984). Existen 4 núcleos de motoneuronas en los segmentos L6 y S1: dorsomedial, ventral, dorsolateral y retrodorsolateral (Schoeder, 1980). Las motoneuronas de estos núcleos inervan a músculos del piso pélvico, entre ellos a los músculos del diafragma pélvico (Breedlove y Arnold, 1980; Jordan y col., 1982; McKenna y Nadelhaft, 1986; Lucio y col., 1990). En rata macho se ha mostrado que un músculo pélvico puede estar inervado por motoneuronas distribuidas hasta en tres núcleos distintos (Lucio y col., 1990). Dimorfismo sexual en los núcleos motores de la médula espinal lumbar se ha descrito en rata (Breedlove y Arnold, 1981). Diferencias significativas entre hembra y macho, con respecto al tamaño y al número de neuronas, se han encontrado en los núcleos dorsolateral, ventral y dorsomedial (Breedlove y Arnold, 1981; Jordan, 1982). Las neuronas del ganglio de la raíz dorsal y de los ganglios de la cadena simpática correspondientes a los segmentos L6 y S1, también muestran diferencias sexuales (McKenna y Nadelhaft, 1986). Se ha mostrado que las neuronas del núcleo dorsolateral y del núcleo dorsomedial, y los músculos que ellas inervan, están bajo control hormonal, y que tal control hormonal es el responsable del dimorfismo sexual (Breedlove y Arnold, 1981).

En otro de mis estudios (Pacheco y col, 1987), analizamos la organización neural de los últimos segmentos sacros y coccígeos de la médula espinal del gato, utilizando el método de la estimulación de raíces dorsales intactas y seccionadas, y del registro de la actividad de las raíces ventrales correspondientes. Encontramos que dichos segmentos de la médula espinal carecen de una organización refleja uniforme. La estimulación supraumbral, en cada uno de los ocho segmentos analizados, produjo respuestas en las raíces ventrales con características específicas a cada segmento. Después de la sección de la raíz dorsal, la respuesta monosináptica mostró un aumento, principalmente en aquellos segmentos que antes de la sección, exhibían un débil reflejo monosináptico. Las respuestas de la raíz dorsal no cambiaron con la sección. Esta falta de uniformidad en la organización refleja puede indicar algunos aspectos de la complejidad anatómica y funcional del área pélvica y caudal. El hecho que hayamos encontrado actividad predominantemente polisináptica en el segmento S2, por la estimulación de la raíz dorsal completa, correlaciona con la falta de respuesta monosináptica, que ocurre cuando se estimula el nervio pudendo (Dubrovsky y Pacheco, 1980; Jolesz y col., 1982; Dubrovsky y col., 1985).

Esta porción terminal de la médula espinal está relacionada a una de las partes más complejas del sistema nervioso autónomo: los plexos abdominales y pélvicos, sitios de convergencia y divergencia de las vías simpáticas y parasimpáticas. De los últimos segmentos lumbares, de los sacros, y de los ganglios autonómicos, se originan las fibras nerviosas de los nervios que se distribuyen sobre las estructuras pélvicas. En mamíferos, los principales nervios son: pudendo, pélvico, hipogástrico y genitofemoral; los primeros tres están relacionados con el plexo pélvico.

En la rata, la mayoría de las células preganglionares simpáticas, se proyectan desde los segmentos espinales lumbares, vía los nervios esplácnicos lumbares, cuyos axones hacen o nó sinapsis con las células posganglionares del ganglio mesentérico inferior. Los axones que no hacen sinapsis, después de cruzar el ganglio viajan por el nervio hipogástrico, que a su vez está constituido por axones de las células postganglionares del propio ganglio mesentérico inferior. Estos axones, pre- y postganglionares se dirigen hacia el ganglio pélvico, más cercano a los órganos efectores.

El resto de las células preganglionares simpáticas, se proyectan caudalmente en la cadena simpática paravertebral formando sinapsis con las neuronas postganglionares de los ganglios lumbosacros de dicha cadena; los axones postganglionares llegan a los órganos pélvicos vía los nervios pélvicos, los cuales también se dirigen hacia el ganglio pélvico.

Las células preganglionares parasimpáticas, se proyectan desde los segmentos espinales sacros, a través de los nervios pélvicos cuyos axones hacen o nó sinapsis con las neuronas postganglionares del ganglio pélvico.

En general, se reconoce que las neuronas preganglionares simpáticas y parasimpáticas están espacial y funcionalmente separadas, sin embargo, ésto parece no ser el caso para la inervación postganglionar a los órganos pélvicos (Jänig, 1987). Existen evidencias de que axones de ambas divisiones autonómicas, forman sinapsis con la misma célula del ganglio pélvico, y que además, solamente algunas neuronas postganglionares inervan directamente a sus órganos blanco, por ejemplo en el caso de la musculatura lisa vascular o de algunos músculos viscerales. Las neuronas postganglionares que no pertenecen a este grupo, ejercen su control a órganos efectores de forma indirecta, a través de otras neuronas periféricas, por ejemplo en el sistema entérico del aparato digestivo o en los ganglios prevertebrales.

El estudio del papel del sistema nervioso en la modulación de la actividad visceral pélvica avanza con mucha dificultad, aunque muchos esfuerzos se le han dedicado. Por ejemplo, Oliver, Bradley y Fletcher han descrito mucha de la inervación visceral en gato y rata (1969a y b, 1970); De Groat y su grupo han estudiado extensamente el papel del sistema nervioso en los procesos de micción, principalmente en gato y mono (1969, 1981, 1983, 1984, 1986; 1989; Roppolo y col., 1985; Thor y col., 1989); así mismo, Jänig ha investigado las aferentes viscerales de los órganos pélvicos, enfatizando en la nocicepción visceral (1986, 1987).

Un factor que ha complicado las investigaciones sobre el significado funcional de esta inervación nerviosa, es la complejidad de los plexos en los animales experimentales. Así, para el estudio de la actividad ganglionar que regula las vísceras pélvicas en mamíferos, los investigadores han buscado el "modelo ideal". Según Purinton y col. (1973) y Gabella (1985), tal modelo ideal se encuentra en el plexo pélvico de la rata que "consiste de un ganglio pélvico único con distintos origenes simpáticos (nervio hipogástrico) y parasimpáticos (nervio pélvico) y nervios postganglionares a las vísceras pélvicas" (Langworthy, 1965). Sin embargo, los estudios de Baljet y Drukker (1979, 1980), los de Hulsebosch y Coggeshall (1982) y los de Dail (1983, 1986, 1989a y b) han revelado una enorme complejidad en el plexo pélvico y los ganglios y vías relacionadas.

La complejidad en la organización de los nervios del área pélvica ha llevado a confusión en la nomenclatura, la descripción de sus orígenes, su distribución sobre estructuras periféricas, la composición de sus fibras, y sobre las funciones en las que participan.

Un ejemplo de tal confusión se puede encontrar en el modelo de la coneja, que a pesar de su importancia en la investigación reproductiva, no existe una explicación integrativa de su organización anatómica relacionada a la conducta reproductiva. Basados en disección gruesa y estimulación eléctrica de músculos estriados en conejas adultas, el propósito del *sexto trabajo* de esta tesis (Martínez-Gómez y col., 1996) fue ayudar a corregir esta carencia descubriendo el origen, inserción y acción general de músculos asociados con el tracto vaginal, junto con las glándulas odoríferas. Comparada a las vaginas de la rata o la gata, la larga vagina de la coneja fue caracterizada por una musculatura compleja bien desarrollada. Los músculos bulboespongioso, isquiocavernoso, constrictor vestibular y constrictor vulvar, pobremente desarrollados o aún ausentes en la rata o la gata, se observaron particularmente conspicuos. Las glándulas odoríferas incluyendo las ahora identificadas por primera ves, glándulas prepuciales, fueron también una característica distintiva del tracto y región pélvicas. Sugerimos que estas estructuras pueden representar especializaciones que subyacen la rápida copulación y el parto breve, así como la bien desarrollada la comunicación química, todas características de la eficiencia reproductiva de la coneja.

Como se puede apreciar de la información precedente, el modelo neuromuscular de la región pélvica es altamente complejo y carece aún de información básica. Diversos cuestionamientos pueden hacerse sobre la dinámica funcional del área pélvica.

El primero atañe a su musculatura. Desafortunadamente, gran parte de la investigación sobre el aparato neuromuscular ha ignorado el consejo de Sappey de hace más de un siglo: "el levator ani es uno de los músculos más estudiados y , al mismo tiempo, del que menos sabemos. La doctrina de continuidad de fibras entre dos o más músculos de acción independiente, ha sido aplicado en varias épocas científicas, y este antiguo error, renovado sin cesar, ha contribuido singularmente a complicar su estudio" (citado por Wendell-Smith y Wilson, 1977). La atención prestada a estos músculos en el hombre, radica en la alta frecuencia conque se presentan desórdenes en su funcionamiento, sobre todo en mujeres. Además, porque se ha reconocido importancia a la musculatura circumvaginal en la respuesta sexual femenina.

El segundo cuestionamiento se remite al estudio de la dinámica funcional del área pélvica en animales. Uno de los problemas atañe a la inervación. En muchas especies, se observa un alto grado de complejidad en la distribución y el origen de los nervios del área pélvica que, como ya se anotó antes, se debe en parte, a la estrecha interacción entre el sistema nervioso somático y el autónomo. En rata existe la confusión conceptual acerca de la anatomía y nomenclatura de los nervios pélvico y pudendo. La mayoría de los autores (Greene, 1935; Kollar, 1953; Carlson y De Feo, 1965; Baljet y Drukker, 1980; McKenna y Nadelhaft, 1986), coinciden en describir el origen del nervio pudendo a partir de los segmentos L6-S1. Pero para describir el origen del nervio pélvico, existen diversas tendencias. Greene (1935) no lo menciona en su anatomía de la rata, que es aún consultada por muchos investigadores, otros lo consideran como una rama del nervio pudendo (Kollar, 1952), o que se origina directamente de los segmentos S1-S2 (Reiner y col., 1980), o que junto con el nervio pudendo, se origina directamente del llamado tronco L6-S1 (Baljet y Drukker, 1980; McKenna y Nadelhaft, 1986). El nervio pélvico ha sido considerado exclusivamente autonómico (Hulsebosch y Coggeshall, 1982), sin embargo se ha descrito (Peters y col., 1987) que también contiene un componente somático el cual, en observaciones preliminares realizadas por nosotros, aparentemente juega un papel muy importante en la dinámica neural del piso pélvico.

La inmensa cantidad de reflejos que se suscitan, por ejemplo, durante la cópula o el parto, requieren para su estudio de información anatómica y fisiológica básica.

Es importante entonces, realizar investigaciones cuidadosas que indaguen específicamente la naturaleza y la dinámica de la actividad de los nervios pélvico y pudendo en relación a la médula espinal y a los músculos del área pélvica. Así mismo, es necesario caracterizar la participación de la musculatura pélvica, lisa y estriada, en las múltiples funciones que en esta área se suscitan. Finalmente, resulta relevante el análisis integrativo de la actividad refleja pélvica reproductiva, posiblemente modulada por las hormonas sexuales y desencadenada en machos y hembras por la estimulación coital.

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Spinal Control of Pelvic Floor Muscles

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A prevalent notion in the literature is that the pelvic floor muscles behave as a unitary mass. We examined this proposition experimentally. In spinal cats, we recorded EMG activity from the following pelvic floor muscles: the sphincter ani externus (SAE), the abductor caudae internus (coccygeus), and the levator ani (pubiocaudalis) muscles. The epaxial sacrocaudalis dorsalis lateralis muscle was also exposed and prepared for recording. Electrical stimulation of S2 ventral roots elicited twitch responses of the sphincter ani externus and of the sacrocaudalis dorsalis lateralis muscles. Stimulation of S3 and Cx1 ventral roots elicited responses in the other two muscles studied, the levator ani and abductor caudae internus. Thus a clear segregation of the segmental motor neuron pools innervating the different pelvic floor muscles was demonstrated. The various muscles of the pelvic floor region could be reflexly activated either individually or as a mass unit depending on the intensity of stimulation. Tactile or electrical stimulation of pudendal regions on either side of the body elicited responses of the sphincter ani externus. In contrast, activation of the levator ani and abductor caudae internus muscles could be lateralized: tactile or electrical stimulation of the dorsolateral surfaces at the base of the tail region elicited ipsilateral responses from these muscles. Section of one pudendal nerve did not alter the level of tonic activity (2 to 4/s) of the sphincter ani externus. However, bilateral section of the pudendal nerve entirely abolished both tonic activity and phasic responsiveness of the SAE without affecting the activity of the levator ani and abductor caudae internus muscles. Pudendal nerve stimulation elicited only polysynaptic reflex responses from S2 ventral roots. The results presented show that the neural apparatus of the striated musculature of the pelvic floor is capable of activating individually the different muscles that make up the system, and that the sphincter ani externus from one side, and muscles that conform the diaphragm pelvis from the other, are subserved by different neuronal circuits.

Abbreviations: SAE—sphincter ani externus, T—threshold.

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INTRODUCTION

In most terrestrial mammals, the caudopelvic musculature can be divided into (i) true sphincters and muscles tethering the sphincters to surrounding structures, the sphincter cloacae group, and (ii) muscles which flank the visceral outlets, bilateral structures attached to the inner aspect of the pelvis anteriorly and laterally, and to the lower segments of the vertebral column posteriorly (48, 49). The levator ani complex, or muscle diaphragm pelvis, is the main component of the second group, which has been referred to as the compressor cloacae group by Wendell-Smith (48). Traditionally, the levator ani complex has been considered to consist of the pubococcygeus, the iliococcygeus, and the coccygeus muscles (41, 45, 48, 49). The puborectalis, a muscle unique to the human species (45), is thought by some researchers to be part of the levator ani complex (35, 45), whereas other researchers consider that the puborectalis and the deep sphincter ani externus (SAE) form an indivisible anatomic and functional unit (32, 41).

Many EMG studies (12, 18, 19, 21, 22, 25, 43, 46), however, have demonstrated that both the puborectalis and SAE muscles show a continuous level of activity which continues even during sleep. The firing discharge rate of these muscles in a resting state is 3 to 4/s, a rate far lower than those observed in typical antigravity muscles such as the soleus, whose discharge rates are 8 to 10/s in the standing posture (47).

From a study of the activity of the SAE and other pelvic floor muscles, Porter (36) concluded that it is of reflex origin, with the afferent impulses arising from the pelvic floor muscles themselves. He called this "the postural reflex of the pelvic floor."

Although the SAE muscle has been extensively studied in man (12, 18, 19, 21, 22, 25, 29, 30, 36, 41, 44) and in experimental animals (5, 6, 22, 42, 46, 50), little is known of the specific behavior of components of the muscle diaphragm pelvis.

A prevalent notion is that the pelvic floor muscles behave as a unitary mass (12, 15, 36, 48, 49). This proposition is difficult to reconcile with the fact that besides being a main support for pelvic viscera, these muscles participate in a number of distinct and complex behaviors, i.e., continence, defecation, and micturition, and sexual and reproductive activities.

We examined the problem of activation of pelvic floor muscles to determine whether they contract as a unit or can be selectively activated. Further, some aspects of the spinal segmental organization of pelvic floor muscles were examined. Preliminary results of this work have been reported (13, 14).

METHODS

Adult cats were anesthetized with ether and after tracheostomy and

The radial artery and vein were cannulated for blood pressure monitoring and i.v. infusion, respectively. Dextran was infused when blood pressure decreased to less than 80 mm Hg.

After transection of the spinal cord, ether was discontinued and the animals were placed in a spinal unit, with the head fixed by a nontraumatic head-mouth piece firmly attached to a rigid bar. The lumbosacral and caudal regions of the spinal cord were exposed by bilateral laminectomy. The skin edges were stitched to the metal spinal unit, and warm (37°C) mineral oil was poured into the pool so formed. The temperature of the pool was maintained with an infrared lamp regulated through a feedback circuit provided by a temperature controller Y.S.I. The dorsal and ventral roots S2, S3, and Cx1 were separated and prepared for recording and stimulation. The pudendal nerve, ipsilateral to the side of the prepared roots, and its hemorroidal branch were dissected out and mounted for electrical stimulation.

The types of afferent fibers stimulated were monitored by recording the afferent volleys from the sacral and caudal dorsal roots. The minimal current necessary to elicit a volley in the roots was taken as the threshold (*T*), and the intensities of subsequent stimulations were expressed as multiples of that value.

Threshold values were usually about 250 to 300 μA for biphasic pulses of 0.05 to 0.1 ms duration delivered at 1/s. Potentials were amplified on 122 Tektronix preamplifiers set at 0.1 Hz (low) and 10 kHz (high) frequency response. After further amplification, potentials were displayed on a 565 Tektronix oscilloscope, and filmed from it with a Grass camera.

Somatic stimulation was effected by brushing the skin with a fine hairbrush; by touching the skin at the base of the tail, pudendal, and upper hind limb regions; and by pinching a fold of skin, gradually increasing the pressure. To study the latency and following frequencies of the response, needle electrodes were inserted through the skin of these regions for electrical stimulation.

The following pelvic floor muscles as described and named in Crouch (11) were exposed: the sphincter ani externus, the abductor caudae internus or coccygeus, and the levator ani or pubiocaudalis muscle. The second and third of these muscles form a vertical pelvic diaphragm which closes off the pelvic cavity (11). As the effects of S2 ventral root stimulation were going to be examined, the epaxial sacrocaudalis dorsalis lateralis muscle (10), which has been described by some authors as the medial division of the longissimus dorsi (38), was also exposed and prepared for recording. Anatomical studies indicate that this muscle is innervated by a separate nerve trunk ascending from the sacral region with a major component from S2 (7).

All muscles were under warm mineral oil in a pool

For EMG recordings, two copper wires, completely insulated except for 1 mm, were threaded through each of these muscles, with the uninsulated section buried in the muscle. Signals were amplified and recorded as described above.

RESULTS

Ventral Root Stimulation. The distribution of spinal motor efferent fibers to the various muscles studied was investigated in eight animals.

Stimulation of S2 ventral roots elicited twitch responses from the SAE in all animals examined (Fig. 1). Only in one instance was a response from the SAE observed with stimulation of S3 ventral roots. Consistent with Bogduk's (7) anatomical studies, stimulation of S2 ventral root also activated the sacrocaudalis dorsalis lateralis muscle (Fig. 1). Stimulation of S3 and Ca1 ventral roots always elicited responses in the other two muscles studied: the levator ani (pubiocaudalis) and abductor caudae internus (coccygeus) (Fig. 1).

Thus the results of ventral root stimulation revealed that different pelvic floor muscles receive their neural motor supply via different spinal segments.

Peripheral Stimulation. The various muscles of the pelvic floor region could be reflexly activated either individually or as a mass unit depending on the intensity of the stimulation.

Natural or electrical stimulation of pudendal regions on either side of the body elicited responses of the SAE. The anal reflex [i.e., contraction of the SAE in response to perianal scratching (22)] when elicited by electrical stimulation of perianal regions had latencies of 7 to 9 ms, (Fig. 2A) and could faithfully follow frequencies up to 6 to 8/s.

In contrast to the bilateral activation of the SAE muscle, activation of the levator ani and abductor caudae internus muscles could be lateralized: tactile or electrical stimulation of the dorsolateral surfaces at the base of the tail region (38) elicited ipsilateral responses from these muscles (Fig. 2A). Muscular responses to the electrical stimuli had latencies of 8 to 10 ms, and the twitch responses could consistently follow frequencies up to 4 to 6/s.

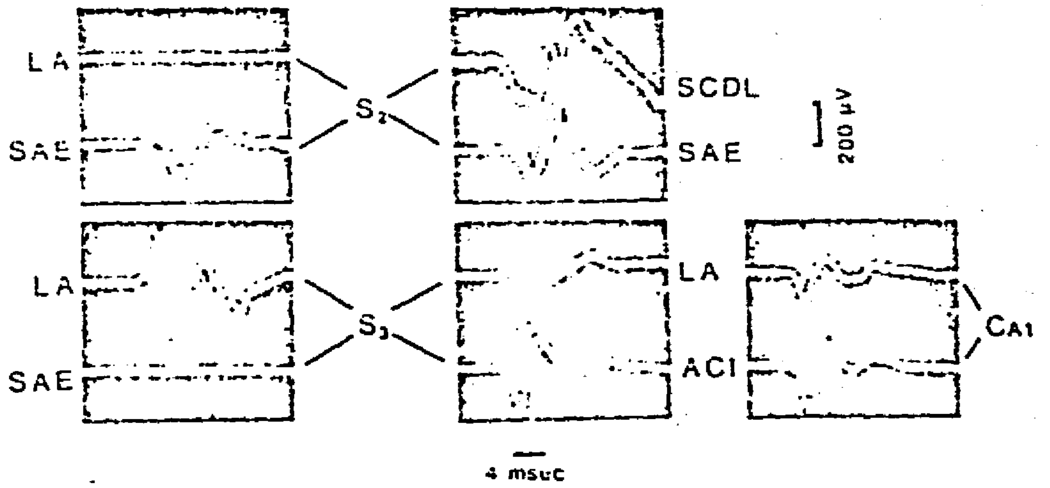


FIG. 1. Electromyograms of twitch responses of caudopelvic muscles to stimulation of sacral (S2, S3) and caudal (CA1) ventral roots. Abbreviations: LA—levator ani, SAE—sphincter ani

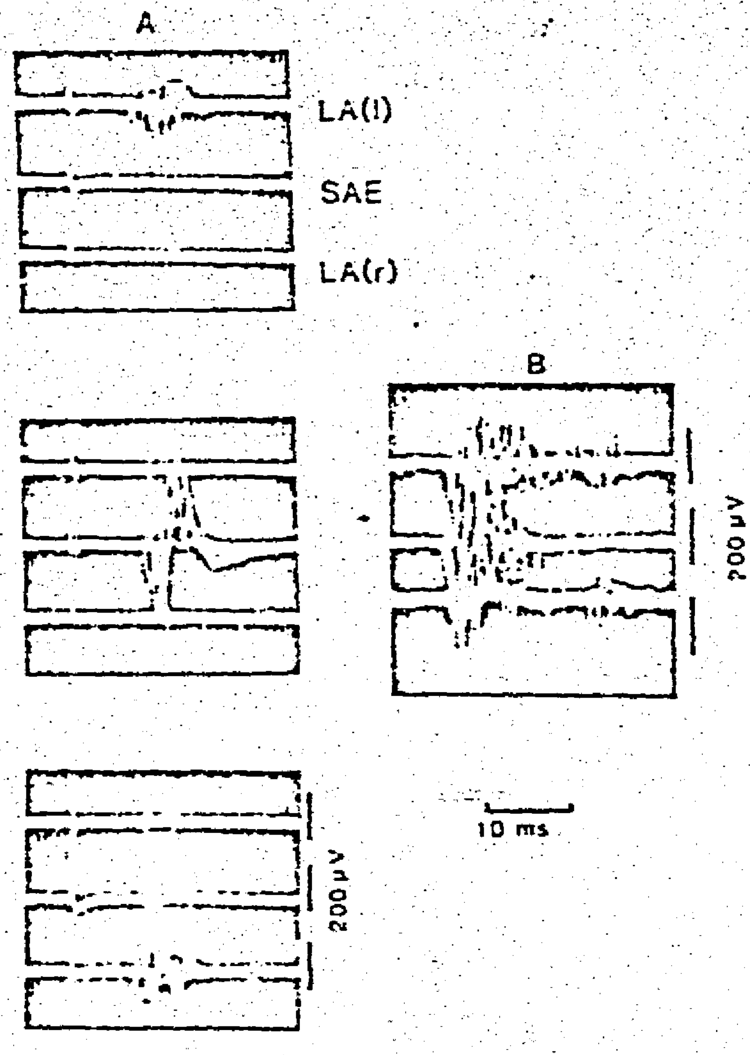
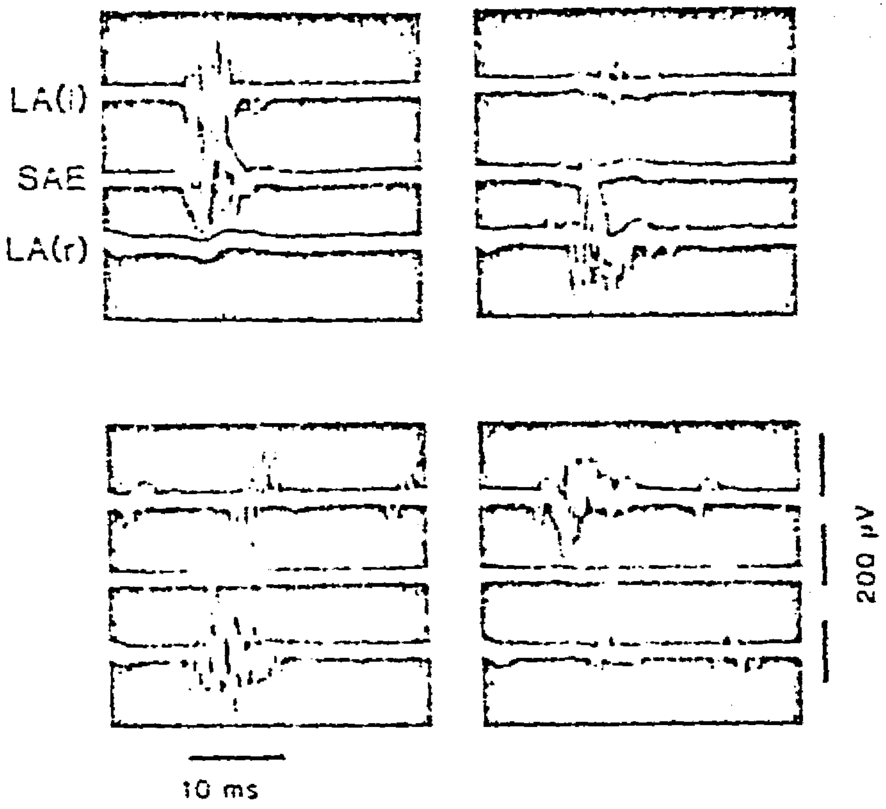


FIG. 2. Reflex activation of pelvic floor muscles. LA(l)—left levator ani, LA(r)—right levator ani; A, individually and B, as a mass unit. Muscle responses displayed in all panels as in upper row left.

Increasing the intensity of stimulation, either by augmenting the pressure applied on the folded skin or the current delivered (to 25 mA), produced simultaneous contraction of all muscles (Fig. 2B).

Transection of one pudendal nerve did not alter the level of tonic activity (2 to 4/s) of the SAE. However, bilateral transection of the pudendal nerve entirely abolished both tonic activity and phasic responsiveness of the SAE without affecting the activity of the levator ani and abductor caudae internus muscles (Fig. 3).

Pudendal Stimulation. The characteristics of the spinal segmental organization of pudendal afferent fibers were investigated in six animals. First, the hemorroidal nerve, a branch of the pudendal nerve that innervates the SAE, was stimulated. Then the pudendal nerve in its entirety was stimulated. Sacral ventral roots S2 and S3 were transected in these experiments, and



:G. 3. Effects of bilateral pudendal transection on reflex activation of pelvic floor muscles.
per row, reflex activation of LA(l) left levator ani, SAE, and LA(r) right levator ani. After
teral pudendal section, lower row, only levator ani muscles were activated.

dendal nerve stimulation elicited only polysynaptic reflex responses. It
s not possible to observe monosynaptic responses (4) to pudendal nerve
nulation either with maximal stimulation or with tetanic priming stim-
tion (500/s biphasic pulses 0.1 ms at 6 *T* for 30 s) (Fig. 4). Because
nosynaptic discharges might have been missed in the entire ventral root
ivity, in three experiments small filament bundles were prepared by
tion of S2 ventral roots, and potentials were recorded from them in
se to electrical stimulation of the ipsilateral pudendal nerve. No
nosynaptic responses were detected under this condition (Fig. 4).
That the preparations studied could sustain monosynaptic ventral root
ponses was verified in all cases by the presence of monosynaptic responses
S2 and S3 ventral roots (latencies of less than 1.2 ms after afferent
leys recorded from the corresponding dorsal roots, high-frequency follow-
more than 100/s, and constant delay) when S2 and S3 dorsal roots were
ectly stimulated (26).

DISCUSSION

Although not demonstrating a selective activation of different pelvic floor
les during different behaviors, the results presented in this paper show

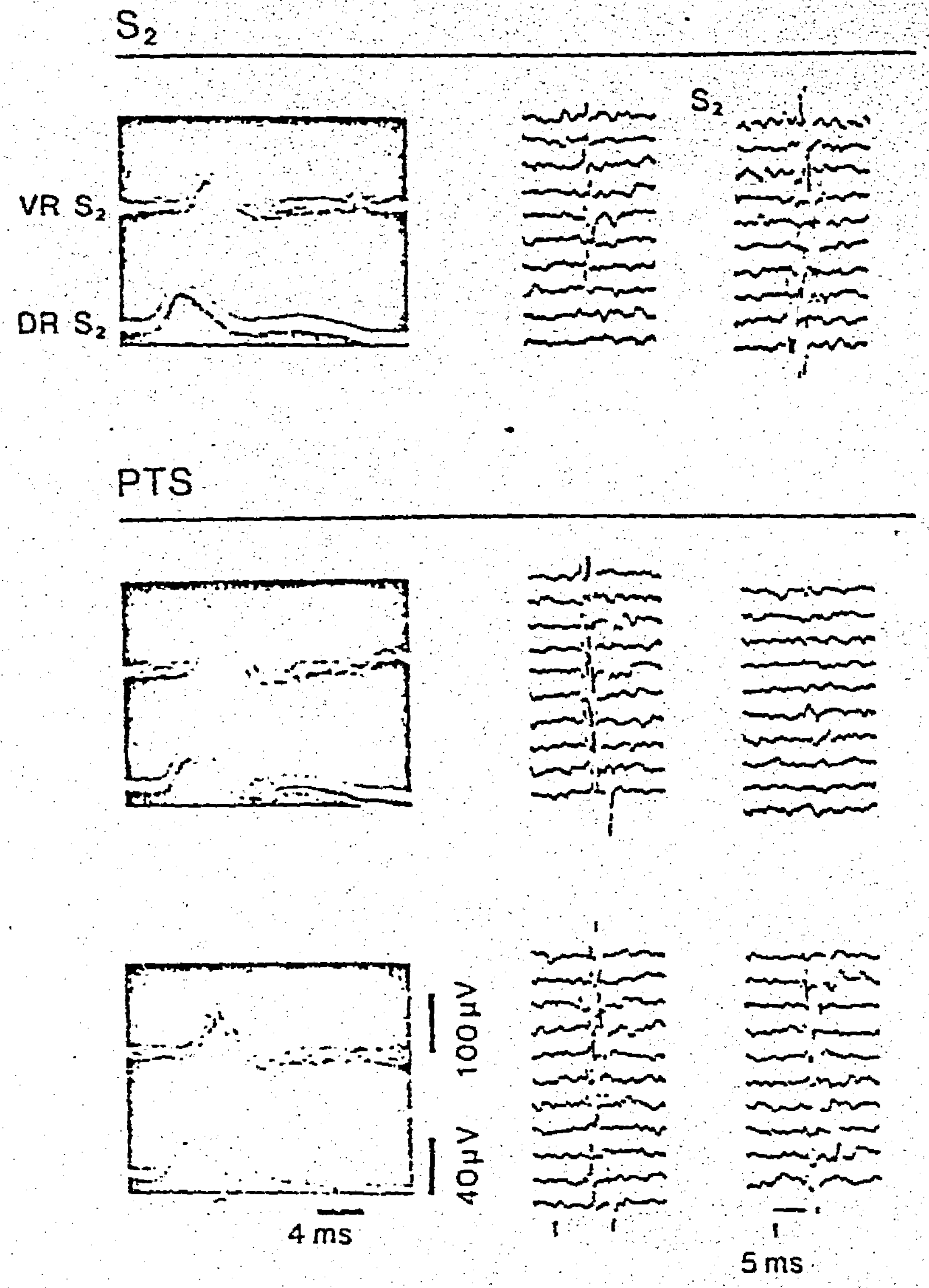


FIG. 4. Ventral root responses to pudendal nerve stimulation. Horizontal upper row: responses of the entire ventral root S2 (VR S2) and from filament bundles of VR S2, middle and right pictures. Dorsal root of S2 (DR S2) recording of afferent volley from dorsal root S2. PTS—posttetanic stimulation. Middle and lower horizontal row, effects of tetanic stimulation (500/s, 0.1 ms, pulses for 30 s) of pudendal nerve on ventral root responses, after 1 and 3 min of stimulation respectively. Arrow, time of test pudendal stimuli. No monosynaptic responses are observed.

Ventral root stimulation or peripheral reflex activation produced a response in the SAE independently of the levator ani and the mammals (47, 48). Furthermore, tonic activity and phasic responses of muscles of the compressor cloacae group were not affected by bilateral pudendal transection, which totally abolished all activity in the SAE muscle.

Our finding that the SAE muscle responded to stimulation of either side of the body is in keeping with results of other authors, Bishop (6), Gunterberg *et al.* (21), Jolesz *et al.* (24), Wunderlich and Swash (50), who also confirmed Sherrington's (42) early (1892) observations on the overlap in the distribution of motor fibers from the right and left pudendal nerves.

In contrast to the SAE responses, responses of compressor cloacae muscles, the levator ani and abductor caudae internus, could be lateralized. Similar results on the lateralization of the innervation of the puborectalis were recently obtained during surgical intervention by Percy *et al.* (35) in a study of the motor nerve supply of pelvic floor muscles in humans. They found that each puborectalis muscle is innervated by direct branches of sacral nerves and that "each side of the puborectalis is supplied only by the nerves from that side." Taken together, these observations indicate that the neural mechanisms subserving control of sphincter cloacae muscles differ from those controlling muscles of the compressor cloacae group.

The pudendal nerves innervate both striated sphincters, anal and urethral, and pudendal skin regions (8, 11, 39). Although the anal and urethral sphincters possess a muscle spindle apparatus (33, 43, 46), no monosynaptic responses were recorded in this study from sacral ventral roots after pudendal nerve stimulation. Monosynaptic excitatory postsynaptic potentials (EPSPs) have been recorded from sacral motoneurons in response to pudendal nerve stimulation (23, 27). However, these EPSPs had low amplitudes (rarely larger than 0.5 mV) and low frequency connectivity; less than half of the motoneurons tested received monosynaptic EPSPs. These factors could account, in part at least, for the absence of monosynaptic ventral root responses to pudendal stimulation in spinal animals (24). Monosynaptic ventral root responses after stimulation of neck muscle afferent fibers are rarely observed in the cat (1, 2, 9, 37) even though the dorsal neck muscles are richly endowed with muscle spindles (20, 40). These muscles, however, display a well developed tonic stretch reflex (3) which allows for integrative neural mechanisms of a far higher order than that displayed in the tendon jerk resulting from a single synchronous input (28).

It is tempting to suggest that in muscles like those in the dorsal neck region that interact with vestibular and oculomotor systems in the coordination of eye-head movements (3), and in muscles like the SAE subjected to multiple influences (e.g., postural, respiratory, as well as the segmental input), the final common paths are heavily dependent on suprasegmental input (19, 27, 29).

Evidence has now accumulated showing that the

(22, 31, 34). As connectivity at spinal, and probably supraspinal levels too (30, 44), is different for the SAE and for the diaphragm pelvic muscle complex, it follows that functional examination of the pelvic floor musculature should sample its different muscle components individually.

To consider an EMG sampling of the SAE activity as representative of the functional state of the entire pelvic floor musculature would be erroneous and misleading. We think that the selective neural connectivity (afferent and efferent) of each pelvic floor muscle should also be taken into account in the design of therapeutic strategies for incontinence using electrostimulation. Therapeutic failure (16, 17) may be more frequently related to diffuse, inappropriate stimulation of the pelvic floor musculature than hitherto suspected. Indiscriminate stimulation can coactivate muscles such as the SAE and levator ani complex, which may have reciprocal patterns of activity, whereby as one contracts, others relax or vice versa (8, 41) under normal physiologic conditions.

The results presented also indicate the need to search for organized functional subsets within large complex muscular groups such as that of the pelvic floor, as has been found to exist in another equally complex and specialized group, that of the dorsal neck muscles (2, 3, 9, 37).

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Effects of Genital Stimulation upon Spinal Reflex Activity of Female Cats under Various Hormonal Conditions[1]

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PACHECO, P., C. BEYER, G. MEXICANO AND K. LARSSON. *Effects of genital stimulation upon spinal reflex activity of female cats under various hormonal conditions.* PHYSIOL. BEHAV. 17(4) 699–703, 1976. — Electrical responses to stimulation of either the medial gastrocnemius or the sural nerve were recorded from the L_7 ventral root in female spinal cats (C_1 – C_2 level). Experiments were performed in 7 intact, 10 ovariectomized, and 8 estradiol treated ovariectomized cats. Stimulation of the sural nerve elicited a two peaked polysynaptic response, and stimulation of the medial gastrocnemius nerve produced a monosynaptic response followed by a complex polysynaptic response. Tactile stimulation of the perivulvar skin area or the neck inhibited the polysynaptic response to both sural and medial gastrocnemius stimulation and facilitated the monosynaptic response to medial gastrocnemius stimulation. Vaginal and cervical stimulation inhibited the polysynaptic response induced by medial gastrocnemius stimulation. Enhancement of the monosynaptic response to medial gastrocnemius stimulation was noted at cessation of cervical stimulation. The effects obtained were independent of the endocrine condition of the animals. The responses here reported are probably related to the posture adopted by the female cat during coitus.

Genital stimulation Spinal reflex activity Ovariectomy Ventral root recording Electrical stimulation

THE ESTROUS cat exhibits a characteristic behavior when mounted by the male. Resting on her chest and forearms, with her pelvis raised and her tail elevated and turned to one side, she executes alternating rhythmic movements of the hindlegs (treading) augmenting in intensity during intromission. The very same behavior can be induced by mechanically stimulating the perineal region and by pressuring the skin of the neck (neckgrip) [7].

Although desensitization has not prevented mating of females [4, 7, 8, 11], importance of genital sensations for regulating female sexual behavior has been suggested, however. Prevention of all responses of a female cat to intromission and mechanical stimulation of the vulva by removing the sacral cord [1] and failure to elicit lordosis in rats by mechanical stimulation of the cervix after pelvic nerve section [5] are observations which show the participation of pelvic nerves in the mating pattern in female animals. More recently it had been suggested [7] that there are several sources of vaginal and uterine sensation during copulation they may be important for mating responses, for example, tactile and deep pressure sensation following insertion of the penis and proprioceptive sensation resulting from muscular contraction of the vaginal and uterine walls.

The neurophysiological mechanisms underlying the behavior pattern exhibited by the cat during mating are not well understood, though it is known that they are integrated at a low level (lower brainstem and spinal cord). Thus Bard [2] induced lordosis and treading in decerebrated cats by stimulating the vulvar or perineal areas.

The present study was undertaken to explore the effects of genital stimulation on spinal reflex activity. Since the crouched position assumed by the cat during genital stimulation presupposes an increased activity of the gastrocnemius muscle, this phenomenon was chosen for electrophysiological investigation. The approach taken was to stimulate electrically the sural and medial gastrocnemius nerves which supply the skin and muscles of the hindleg and to record the resulting electrical activity from lower lumbar ventral roots before, during and after neck and genital stimulation under various endocrine conditions.

METHOD

Animals

Animals used were 25 adult female cats purchased locally. They were used and treated as follows: (A) 7 intact, (B) 10 ovariectomized and (C) 8 ovariectomized and injected with 10 μg of estradiol benzoate daily for 5 to 18 days prior to recording.

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Surgery

Surgery was carried out under ether anesthesia. The spinal cord was transected at the C_1 – C_2 level (carotid and vertebral arteries were not clamped). After spinal cord transection artificial respiration was installed and the anesthesia discontinued. The lumbar and sacral segments of the spinal cord were exposed, and the left L_7 – S_1 ventral roots cut and prepared for recording. Both medial gastrocnemius and sural nerves were dissected on the left side and prepared for electrical stimulation. At times both left and right pudendal nerves were dissected and prepared for recording or sectioning. In order to eliminate afferent influences from other nerves, the left sciatic trunk was sectioned below the ramification of the medial gastrocnemius. The nerves and the exposed spinal segments were placed in a mineral oil bath at a constant temperature.

Procedure

In all experiments both the efferent and afferent volleys were recorded by means of bipolar silver electrodes (4 mm separation). The recording electrodes were connected to Tektronix 122 preamplifiers and the potentials visualized in a Tektronix 502 Dual-beam oscilloscope. A Grass 4C kymograph camera was used to photograph the responses. Afferent volleys on the pudendal nerve were recorded in 6 animals, two from each experimental group. Ventral root recordings, however, were done in all the animals used in this study.

Electrical stimuli to the medial gastrocnemius and sural nerves were produced by a Grass S4 stimulator connected to a Grass SIU5 isolation unit. The intensity of the 0.1 msec pulses was modulated according to the amplitude and the configuration of the monosynaptic and polysynaptic responses of the ventral roots. Thus, when medial gastrocnemius was stimulated the intensity of the pulses was raised until both the monosynaptic and the polysynaptic responses responses were clearly obtained. Two peaked polysynaptic response which was usually obtained by stimulation of the sural nerve was used as criterium to select appropriate intensity during the stimulation of this nerve.

Stimulation of the genital area was performed in two ways: (1) light touch and tapping of the perivulvar skin by the finger; (2) inserting a probe into the vagina, slightly pressing it against the clitoris and/or the cervix. In addition, a neckgrip similar to that occurring when the male mounts the female was simulated by pressuring the fur of the neck. The effect of tactile stimulation of the right hindleg in the spinal responses was also studied for comparison purposes.

Since the complexity of the polysynaptic response obtained by stimulation of both sural and medial gastrocnemius nerve made it impossible to use peak height as a measure of reflex size, all observations were made in terms of qualitative aspects. These aspects will be described and illustrated with figures which are representative examples of the effects observed in all the animals. However, the monosynaptic response elicited by the medial gastrocnemius nerve stimulation was quantified (Table 1) using the peak height as a measure of reflex size.

After the completion of the experiment the animals were killed by an overdose of Nembutal.

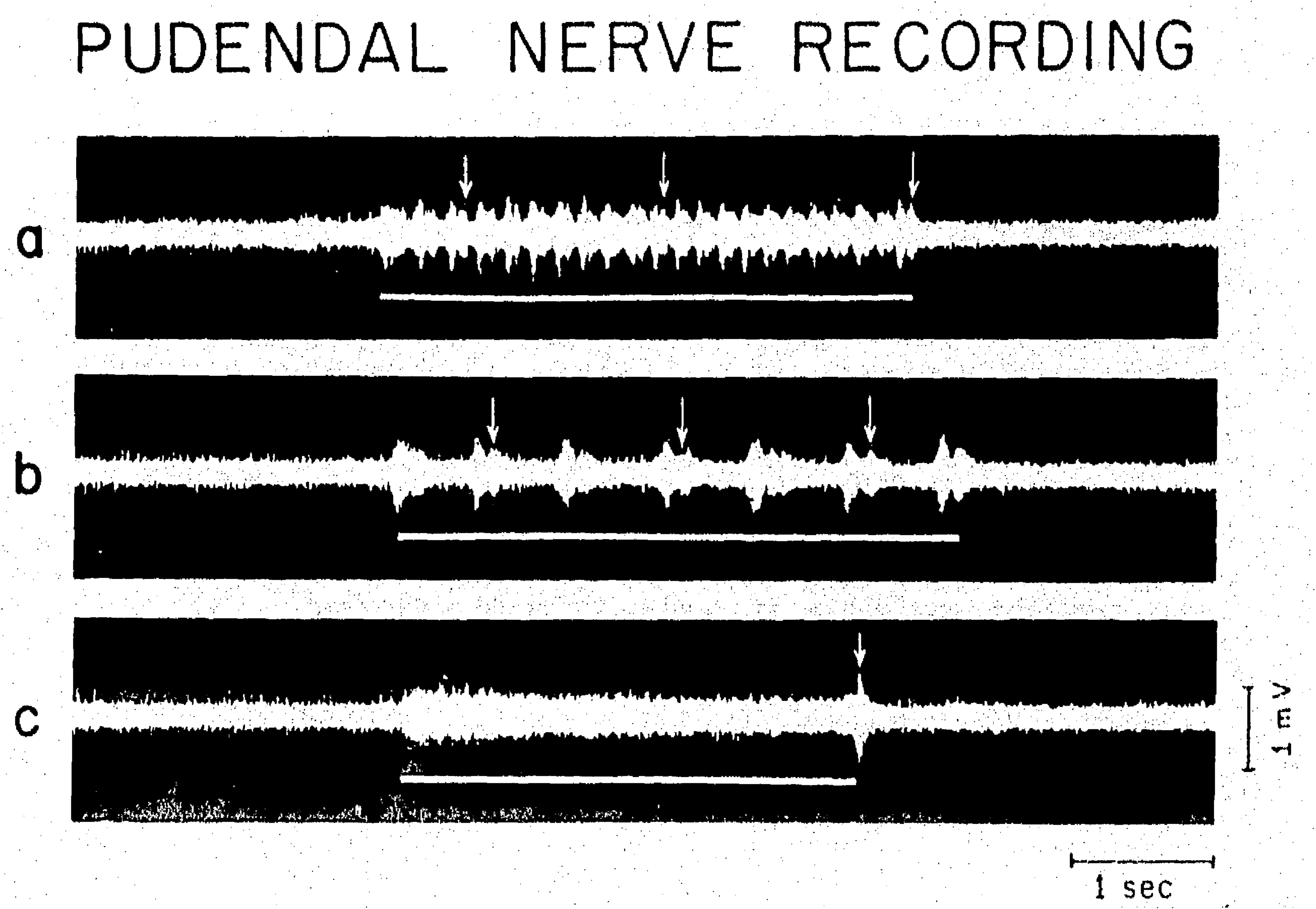

FIG. 1. Response of the pudendal nerve to tapping of the perivulvar skin area. "On" and "off" responses produced by tapping at a rate of approximately (a) 5, (b) 2 per sec or (c) a continuous pressure applied to the perivulvar skin. Arrows point out "off" responses.

TABLE I

CHANGES IN THE MONOSYNAPTIC RESPONSE FOLLOWING MEDIAL GASTROCNEMIUS ELECTRICAL STIMULATION. THE SIZE OF THE MONOSYNAPTIC RESPONSE WAS ASSUMED TO BE 100 PERCENT, AND THE PERCENTAGE OF CHANGE WAS CALCULATED AS A MEASURE OF THE DEGREE OF FACILITATION (+) AND DEPRESSION (−) OCCURRING DURING VARIOUS TYPES OF STIMULATION

	Cat	Days of Ovariectomy	Days of treatment	Tapping Percent change	Neckgrip stimulation Percent change	Vaginal stimulation Percent change	Cervix Stimulation Percent change	Contralateral leg stimulation Percent change
Intact	4	—	—	200 +	100 +			
	19	—	—	300 +	200 +	0	20 −	100 −
	20	—	—	300 +	300 +	100 +	10 −	80
	21	—	—	100 +	150 +	100 +	100 −	100 −
	22	—	—	200 +	100 +	0	0	
	24	—	—	20 +		75 −	20 −	
	25	—	—	250 +	150 +	100 +	10 +	
Ovariectomized	5	10	—	150 +	60 +	0	0	
	6	12	—	50 +	0	90 −	90 −	
	7	18	—	100 +	100 +	20 −	40 −	
	8	17	—	50 +	70 +	80 −	60 −	
	14	10	—	20 +	50 +		50 −	
	16	35	—	100 +	100 +	50 −	20 −	80 −
	26	51	—	300 +	400 +	20 +	150 +	
	27	48	—	20 +	0	80 −	0	
	28	64	—	100 +	100 +	50 −	0	
	29	69	—	100 +	10 +	0	50 −	
Estrogen treated and Ovariectomized	10	22	10	200 +	200 +	100 +	150 +	
	11	32	13	300 +	200 +	100 +	20 +	
	12	21	9	150 +	150 +	0	0	
	30	36	8	150 +	300 +	100 −	50 −	
	31	95	14	200 +	100 +	0	0	
	32	40	15	100 +	25 +	0	0	
	33	44	18	100 +	0	0	0	
	34	60	5	75 +	25 +	0	0	

RESULTS

Pudendal Nerve Recording

Tapping of the perivulvar skin increased the firing of the pudendal nerve as shown in Fig. 1. Two types of responses were observed: a, brief bursts of "on" impulses, and, b, shorter bursts of "off" responses (arrows, Fig. 1). The length of the "on" and "off" responses varied with the frequency of tapping (Fig. 1a and b). When tapping was performed at an approximate rate of 8 per sec an almost steady discharge of impulses was produced. When instead of tapping, a sustained pressure was applied to the perivulvar skin, a continuous "on" response with a tendency to diminish (adaptation) was recorded, followed by an "off" response when the pressure was discontinued (Fig. 1c).

Ventral Root Recording after Electrical Stimulation of the Sural and the Medial Gastrocnemius Nerves

On stimulation of the sural nerve a complex polysynaptic response was recorded from the L_7 ventral root. However, as seen in Figs. 2a and 3, a two peaked polysynaptic response was the characteristic pattern of this long latency response (10 msec). Latencies were measured from the end of the stimulation artifact to the beginning of the ventral root response. Tapping (Fig. 2a) and cervical or vaginal stimulation (Fig. 3) consistently inhibited in all the 25 animals studied the second peak of the polysynaptic response to sural nerve stimulation.

Stimulation of the medial gastrocnemius nerve produced a 5 msec monosynaptic response followed by a 12 msec polysynaptic response as shown in Figs. 2b and 3. Tapping (Fig. 2b) and cervical or vaginal stimulation (Fig. 3) produced also an inhibition of the polysynaptic response. Additionally tapping increased the height of the monosynaptic response to medial gastrocnemius stimulation (Fig. 2b). The size of the monosynaptic response was compared before and during tapping and the percentage increase was calculated. As shown in Table 1 the increase varied between 20 and 300%. No differences could be detected between the effects obtained in intact and ovariectomized cats (U = 15.5, NS Mann-Whitney U test) and between intact and ovariectomized estrogen treated cats (U = 21.5, NS).

Stimulation of the vagina or cervix caused variable effects. Mostly, the monosynaptic response was depressed (Fig. 3) but in some cats, facilitation occurred as shown in Table 1. A clear rebound facilitatory effect was consistently noted at the end of the cervical stimulation. This rebound effect also appeared in cases where no preceding depression of the monosynaptic response was observed.

Neck stimulation caused also facilitation of the monosynaptic response (Table 1). No differences in the response were observed under the various endocrine conditions

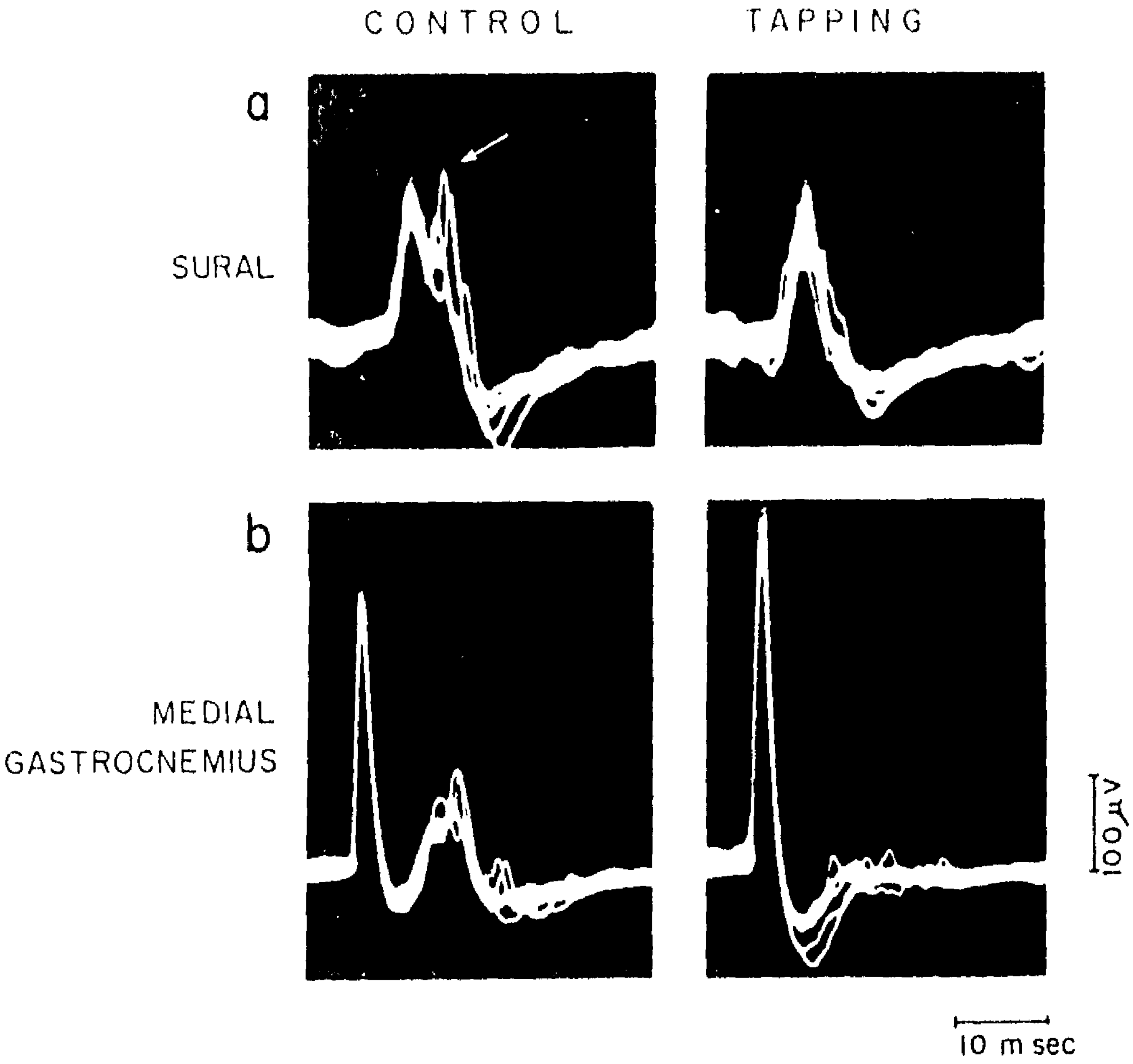

FIG. 2. Recording from the L_7 ventral root to sural (a) and medial gastrocnemius (b) electrical stimulation without (control) and with (tapping) stimulation of the perivulvar skin area. Note inhibition of the second peak of the polysynaptic response of sural during tapping, and facilitation of the monosynaptic accompanied by inhibition of the polysynaptic response of medial gastrocnemius during tapping. Arrow indicates second peak of the sural polysynaptic response. Recordings represent 5 superimposed trials.

analyzed (ovariectomized vs intact, U = 18, NS; ovariectomized estrogen treated vs intact, U = 27, NS).

Stimulation of the contralateral hindleg by light touching produced a strong inhibitory effect on the monosynaptic response. A rebound effect was also observed when the stimulation was discontinued.

DISCUSSION

Sherrington [12] stated as a general rule that stimulation of a cutaneous nerve excites the motoneurons of flexor muscles and inhibits the motoneurons of extensor muscles. Yet, some exceptions have been noted. Thus, Hagbarth [9] found that stimulation of the skin area over the extensor muscle itself activated the motoneurons of that particular extensor muscle. Since stimulation of the skin of the genital area resulting in intense activation of the pudendal nerve facilitated the monosynaptic response of medial gastrocnemius motoneurons, present findings provide another exception to Sherrington's rule.

Komisaruk and Larsson [10] found that vaginal and rectal stimulation blocked the withdrawal reflex exhibited in response to foot pinch by rats. Our results suggest that this effect is due to an inhibition of spinal interneurons since genital stimulation blocked the polysynaptic response provoked by stimulation of both cutaneous and muscle nerves.

The female cat assumes a crouching position when mounted by the male. To maintain this position and support the male, a strong tonic contraction of the gastrocnemius muscle in needed. In fact, this hypothesis is supported by our findings which showed that this contraction is facilitated by sensory stimulation of the genital skin area or by a neckgrip.

The pudendal nerve recording shows how the sensory stimulation to the genital skin area could provoke this facilitatory influence. The short "on" and "off" responses could be responsible for this effect. When, instead of tapping, continuous pressure upon the genital skin area was applied, a prolonged "on" response with a clear tendency to adaptation was observed. The "off" response, however, was short. Thus, it can be assumed that during mating the male cat provokes short "on" and "off" responses upon the pudendal nerve. The gastrocnemius muscle contraction involved on the lordosis behavior is then facilitated.

The tendency for some female cats to maintain lordosis after the withdrawn of the glass-rod used for artificial stimulation of the urogenital sinus and vagina [7], can be

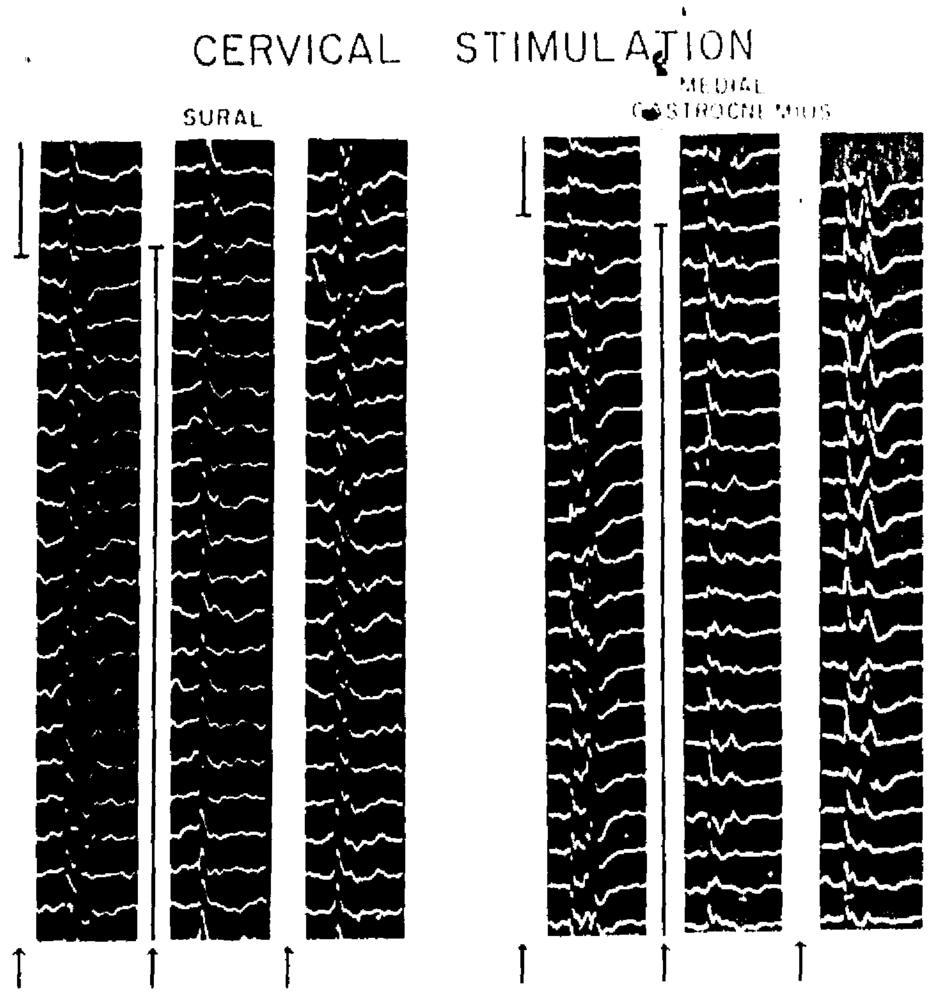

FIG. 3. Continuous recording from L_7 ventral root following sural and medial gastroenemius electrical stimulation without and with (black line) cervical stimulation. Note inhibition of the second peak of the polysynaptic response of sural. Note also the inhibition of both the monosynaptic and the polysynaptic response of medial gastroenemius during cervical stimulation. Recordings start at the base of each figure as arrows indicate.

related with the clear rebound facilitatory effect of the monosynaptic response which was consistently noted at the end of the cervical stimulation.

The present studies were performed exclusively on female cats. It would be interesting to know if the male cat shows a similar response to genital stimulation as the female, particularly in view of the different positions assumed by the male and female cat during mating.

Bard [2] and Dempsey and Rioch [6] reported that the spinal reflexes associated with estrous behavior can be easily evoked in both estrous and anestrous cats. Moreover, Komisaruk and Larsson [10] observed that blockage of the withdrawal reflex can be accomplished in both intact and ovariectomized rats. The finding that the changes elicited by genital stimulation were independent of the endocrine condition of the animal (estrous or anestrous, intact or ovariectomized) agrees with the above mentioned results, and indicates that the well known alteration in sexual responsiveness following estrogen administration are due to action of the hormone at higher nervous levels.

The different effects obtained by stimulation of the various regions of the genital tract may be due to their differences in neural innervation. Thus, the pudendal region as well as the vagina receives its main neural supply from the pudendal nerve, while the cervix is innervated by the pelvic and hypogastric nerves [3].

In some preliminary experiments the pudendal nerve was cut bilaterally and a diminished though not complete blockage of the effects induced by tapping was observed.

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Somato-motor components of the pelvic and pudendal nerves of the female rat

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The efferent innervation of the pelvic and pudendal nerves was characterized in this study by identifying the muscles activated by electrical stimulation of the nerves distal to the point at which they bifurcate from the L_6–S_1 trunk. Pelvic nerve electrical stimulation produced EMG-monitored contraction of the ipsilateral ilio- and pubococcygeus muscles, which was abolished by cutting one ('muscular') branch of the bifurcated nerve. (This 'muscular' branch receives proprioceptive input activated by tail displacement, whereas the other, 'viscero-cutaneous' branch receives sensory innervation from the midline perineal region.)Pudendal nerve electrical stimulation produced contraction of the coccygeus, external anal sphincter, and ischiocavernosus muscles. Movements of the orifice and wall of the vagina were directly visualized during electrical stimulation of the two nerves. Intravaginal pressure measured by balloon was increased by pelvic nerve stimulation and decreased by pudendal nerve stimulation. Reflexive contraction of the ilio- en pubococcygeus muscles was produced by mechanostimulation of the perineum, clitoral sheath and distal vagina. This response was abolished by gentle cervical mechanostimulation. One implication of this finding is that passage of the fetuses through the cervix during parturition may relax the ilio- and pubococcygeus muscles, thereby facilitating delivery.

INTRODUCTION

The pelvic and pudendal nerves contain sensory components that play an important role in reproduction. In female rats, the pelvic nerve receives afferent innervation in part from the vagina and perigenital skin[35] and cervix[24], and transection of this nerve in rats blocks pregnancy[19] and pseudopregnancy[6], probably by preventing prolactin release in response to mating[42]. Pelvic neurectomy also blocks mating-induced ovulation in pentobarbital-blocked rats[13], indicating that the increase in plasma luteinizing hormone in response to mating[31] also may be mediated by activation of this nerve. Vaginal mechanostimulation, which activates pelvic nerve fibers[24], facilitates the lordosis response[20], induces sexual receptivity[38] in rats, and modifies spinal[15,21,31] and cranial[21] reflexes.

The pudendal nerve receives sensory innervation in part from the perigenital skin and clitoris. The size[24] and sensitivity[25] of the sensory skin field of the pudendal nerve is increased by estrogen treatment or during the behavioral estrus phase of the estrous cycle[1] and consequently may also play a role in regulating sexual receptivity in female rats.

While the visceral-motor role of the pelvic nerve has been studied recently[9,10,17], less attention has been paid to the somatic efferent functions of this nerve.

The pelvic nerve in the rat contains parasym-

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athetic[30] and sympathetic[10] components, but its fferent distribution beyond the pelvic plexus[28] has ot been completely determined. In cat, the pelvic erve carries parasympathetic preganglionics to the rinary bladder[9,10,17,32,33]; its electrical stimulation roduces contraction of the detrusor muscle in the ladder[27] and it forms the efferent part of some omato-vesical reflexes[40]. The pelvic nerve also istributes fibers to the urethra[3].

The pudendal nerve contains several somatic fferent branches that distribute to the external anal nd urethral sphincters in the rat[30] and cat[4]. In the t some branches of this nerve are purely cutaneous nsory[8]. In cattle and sheep the pudendal nerve pplies the coccygeus, retractor ani, external anal hincter, retractor penis, ischiocavernosus and bul- ocavernosus muscles[29]. In the female rat it provides series of delicate fibers that innervate the coccy- us muscle[37] and the extremely fine ischiocaver- osus muscle[30]. In male rats, pudendal nerve effe- nts also innervate the bulbocavernosus muscle[30,39] lso referred to as the bulbospongiosus muscle) and e ischiocavernosus muscle[30].

The objective of the present study was to further aracterize the efferent innervation of the pelvic d pudendal nerves in the female rat, by identifying e muscles activated by electrical stimulation of the rious components of these nerves. The branch of e pelvic nerve that receives afferent innervation of e perigenital skin was also identified.

ATERIALS AND METHODS

Intact virgin female Sprague–Dawley rats (250– 0 g) were used, and since qualitative rather than antitative effects of the stimuli were studied, we d not relate the effects to the estrous cycle. All rats ere anesthetized with urethane (1.6 g/kg, i.p.). pplemental doses were administered when neces- ry.

Gross anatomical dissections were performed on 5 ts in order to identify the pelvic viscera, muscles, d course of the nerves innervating the pelvic gion. To facilitate the identification of the pelvic rve, the Carlson and DeFeo[6] description was used. natomical drawings and photographs of the dissec- ns were prepared using a Zeiss surgical micro- ope.

In another 5 rats, to facilitate the identification of the striated muscles controlled by the pudendal and pelvic nerves, electromyographic (EMG) recordings from the muscles were obtained following electrical stimulation of the nerves or their branches. The electrical stimulation of the nerve was performed through bipolar silver chloride electrodes connected to a Grass SIU isolation unit that was activated by a Grass S48 stimulator. Pulses (0.1 ms duration) at 0.5/s were typically used. The EMGs were recorded from 0.1 mm diameter stainless-steel wires inserted into the muscles and connected to Grass 7P3 wide-band AC preamplifiers. The EMGs were displayed on a Tektronix 564 storage CRO. In these 5 animals, in order to measure intravaginal pressure changes during the electrical stimulation of the nerve, a rubber balloon attached to a pressure transducer (Statham) was inserted into the vagina. In another five rats, reflex EMG activity was obtained using mild compression by small blunt forceps on external structures or a blunt probe inserted into the vagina. The Zeiss surgical microscope was also used to directly visualize the vaginal wall (luminal as well as peritoneal surfaces) during nerve stimulation.

Recordings of the electrical activity from the left pelvic nerve of four rats was performed via bipolar silver chloride hook electrodes connected to Grass 7P3 preamplifiers. The responses were displayed on a Tektronix 564 storage CRO. The electrodes were placed on the nerve after its separation from the L_6–S_1 trunk.

RESULTS

Gross anatomy

The pudendal and pelvic nerves travel mainly in spinal roots L_6 and S_1, but they may also travel in spinal roots L_3–L_5 via anastomoses with L_6 and S_1 (Fig. 1A). Spinal roots L_6 and S_1 form a trunk in which the principal component was observed to be L_6 in 12/15 cases (Fig. 1A) and S_1 in 3/15 cases (Fig. 1B). The L_6–S_1 trunk, which is fused for a distance of 5–7 mm, bifurcates into the pelvic nerve and pudendal nerve. Based on gross anatomical observation, most of the fibers of the pelvic nerve appear to originate in S_1.

Pelvic nerve. After its separation from the L_6–S_1

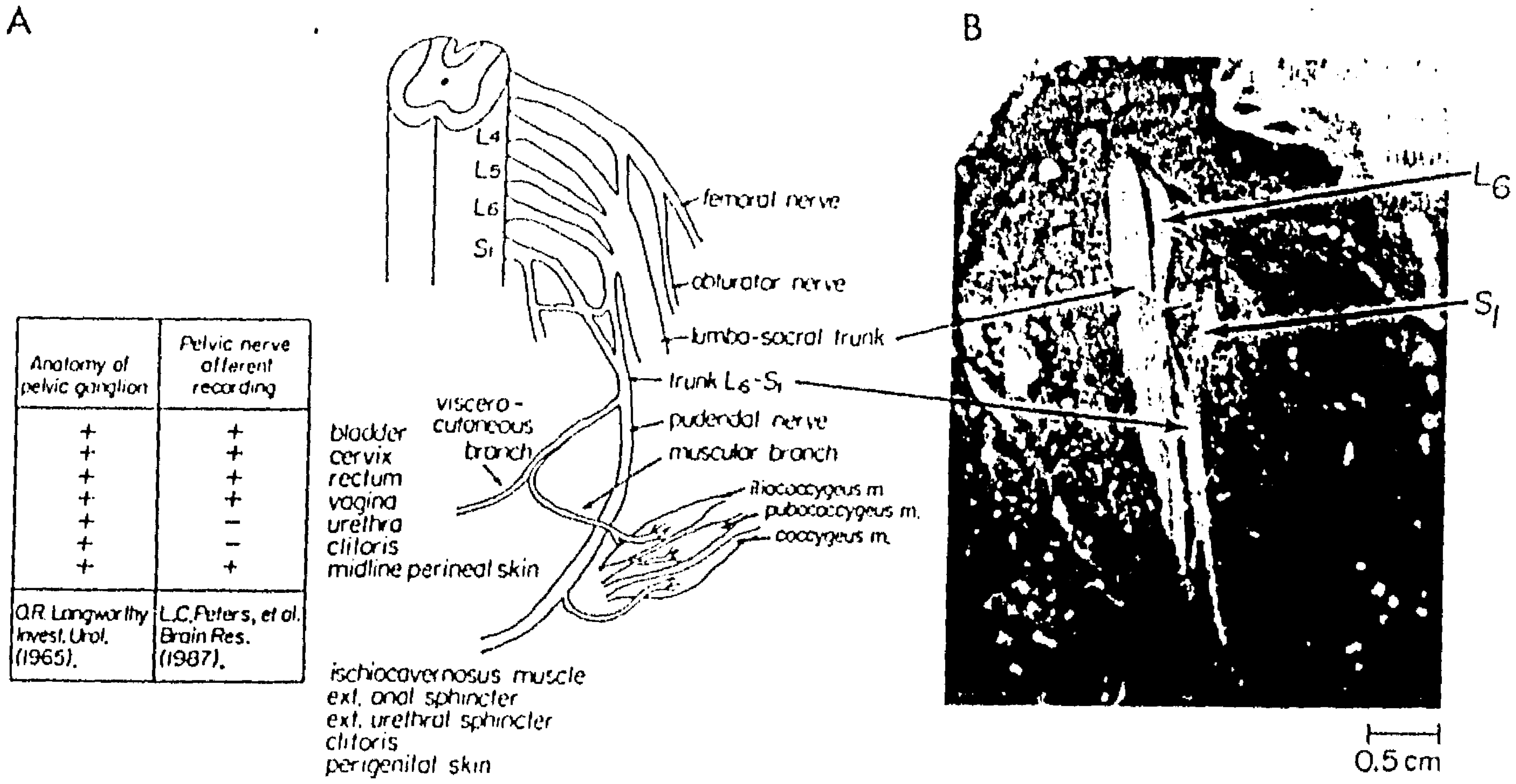
trunk, the pelvic nerve travels caudally approximately 10 mm in a dorsal to ventral direction until it arrives at the medial side of the internal iliac vein. Here a bifurcation occurs. In order to visualize this bifurcation, it was necessary to cut the uterovesical vessels (Fig. 2). One branch of the bifurcation travels to the midline of the body. This consists of 3 bundles that innervate the structures listed in Fig. 1A, and since the branch receives sensory input from skin as well as viscera (see below) we refer to it as the viscero-cutaneous branch. The other branch is the muscular component of the pelvic nerve which is more compact and travels caudally, bifurcating after 5 mm. One branch of the muscular bifurcation innervates the iliococcygeus muscle and the other travels more caudally, innervating the pubococcygeus muscle.

Pudendal nerve. After separating from the pelvic nerve, the pudendal nerve travels caudally and at the level of the medial side of the internal iliac vein bifurcates. The smaller branch travels laterally, bifurcating after 3–4 mm. The two bundles penetrate the coccygeus muscle and one of them joins the other pudendal bundles more caudally. The larger branch travels caudally and after 5 mm divides into three branches. A small branch, the most medial, anastomoses with the lumbosacral trunk (that becomes the sciatic nerve). The other small branch travels caudally into the ischiorectal fossa to the perineal skin. The largest of these 3 branches is the branch to the clitoral sheath. When it reaches the clitoral sheath it divides into several branches that are indistinguishable from connective tissue and from the extremely fine ischiocavernosus muscle[30] that inserts on the crus clitoridis. The other structures related to the pudendal nerve and listed in Fig. 1A were not considered in the present anatomical observations.

EMG of the pelvic muscles

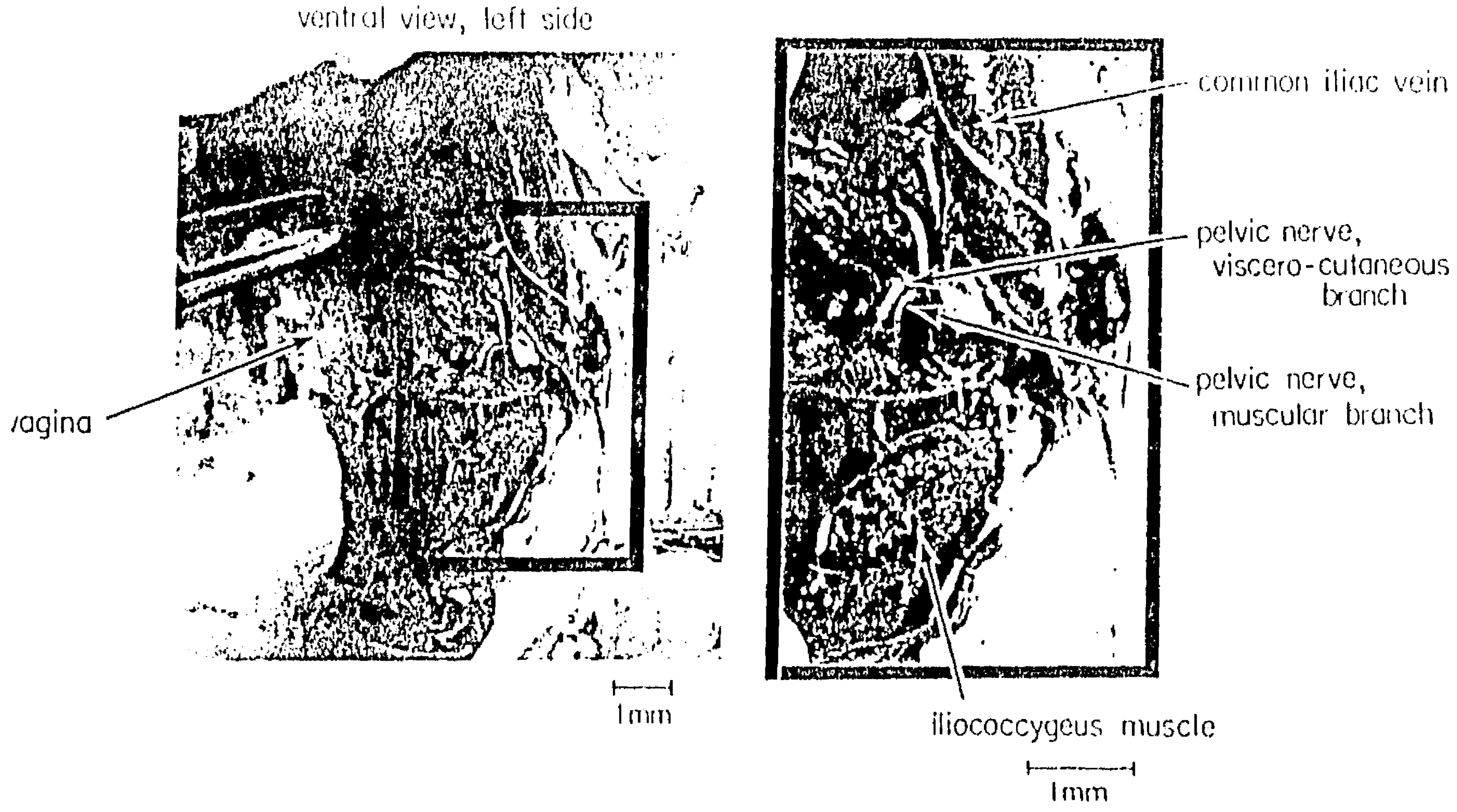
To facilitate the identification of the striated muscles related to the two nerves, EMG recording of the iliococcygeus, pubococcygeus, coccygeus, external anal sphincter, and ischiocavernosus muscles were performed during electrical stimulation of both pelvic and pudendal nerves near their origin from the L_6-S_1 trunk.

Pelvic nerve electrical stimulation elicited contrac-



Anatomy of pelvic ganglion	Pelvic nerve afferent recording
+	+
+	+
+	+
+	+
+	−
+	−
+	+
O.R. Langworthy Invest. Urol. (1965).	L.C. Peters, et al. Brain Res. (1987).

Fig. 1. A: schematic representation of the origin in female rat of the pelvic and pudendal nerves from the lumbo-sacral trunk and from a branch of S_1. The inset summarizes the structures innervated by the pelvic ganglion based on anatomical evidence, and based on recording of pelvic nerve afferent activity. Nerves on left side are shown. B: photograph of a variation in the branching of the pelvic and pudendal nerves. Nerves on right side (different animal) are shown. See text for further explanation.



ig. 2. Left and right panels at low and high magnification, respectively, showing the somatomotor branch of the pelvic nerve that pplies the iliococcygeus and pubococcygeus muscles.

on of the ilio- and pubococcygeus muscles. The
MG activity started approximately 1 ms after the
imulus in both muscles. Sectioning the muscular
ranch of the nerve abolished EMG activity, while
ctioning of the viscero-cutaneous branch did not.
he electromyographic components of the pubo-
ccygeus muscle are less complex than those of the
ococcygeus muscle (Fig. 3). The EMG of the
ococcygeus muscle consists of a slow wave that is
llowed by 4–5 rapid components. This slow wave
obtained even at low intensity nerve stimulation
ig. 4).

Pudendal nerve electrical stimulation produced
ntraction of the external anal sphincter, coccygeus
d ischiocavernosus muscles, or movement of the
itoral sheath and perineal wall. The EMG activity
the external anal sphincter and the ischiocaver-
sus muscles appeared approximately 1.5 ms after
e stimulus (Fig. 5). If the tail was manually
splaced to the side contralateral to the nerve, the
imulation returned the tail to the midline, although
il movement was not elicited from a midline
arting position.

Vaginal muscular activity

Movements of the orifice and wall of the exposed vagina were visualized using the surgical microscope during electrical stimulation of the two nerves. Pudendal nerve stimulation produced lateral dis-

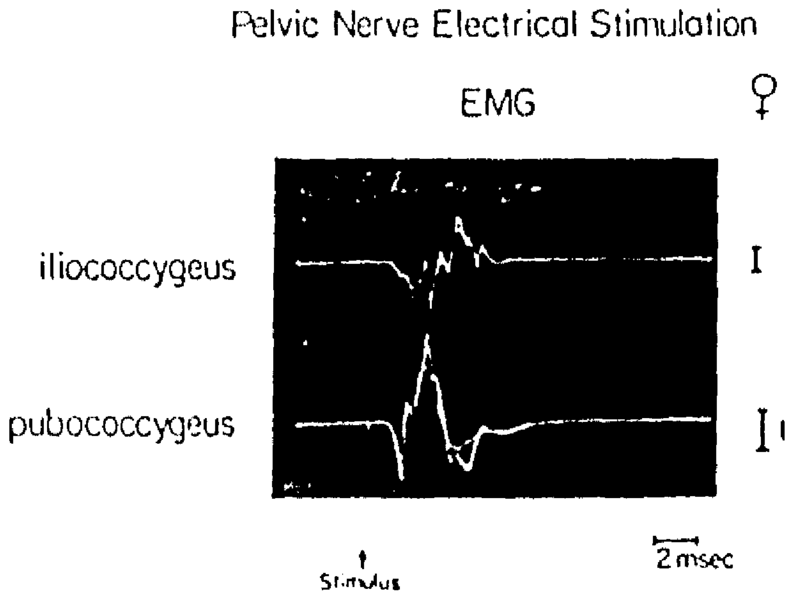


Fig. 3. EMG activity in the iliococcygeus and pubococcygeus muscles evoked by electrical stimulation of the somatomotor branch of the pelvic nerve. Sectioning of this branch abolishes this evoked EMG activity.

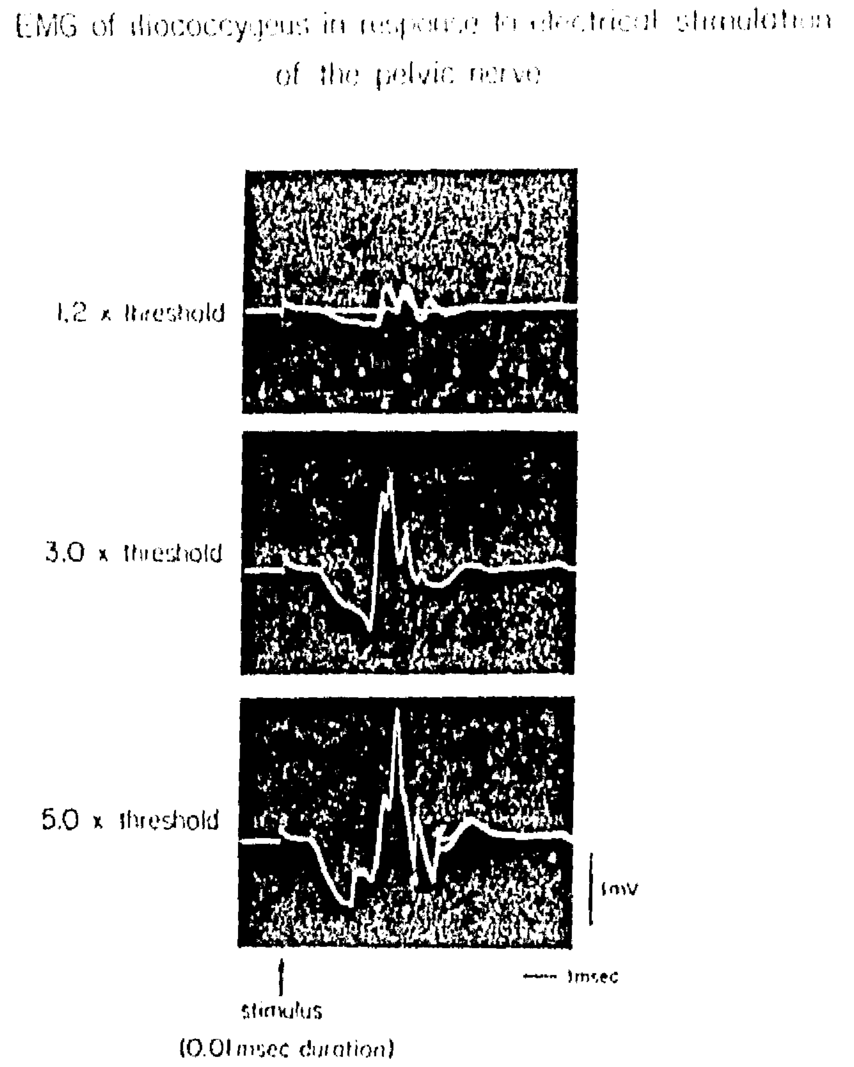


Fig. 4. EMG of iliococcygeus in response to electrical stimulation of the pelvic nerve.

placement of the vaginal orifice ipsilateral to the stimulated nerve, combined with closure of the anal orifice. Pelvic nerve stimulation produced medial displacement of the middle third of the lateral part of the vaginal wall, observed through the dissecting microscope, that was positioned over the abdominal surgical opening. Similarly to the pudendal nerve stimulation (see above), if the tail of the rat was positioned on the midline, the electrical stimulation of the pelvic nerve did not produce movement of the tail. However, if the tail was manually displaced to the contralateral side of the nerve, the stimulation returned the tail to the midline. Furthermore, upon cutting the pelvic nerve with a scissors (5 additional rats), contraction of the tail toward the ipsilateral side was observed.

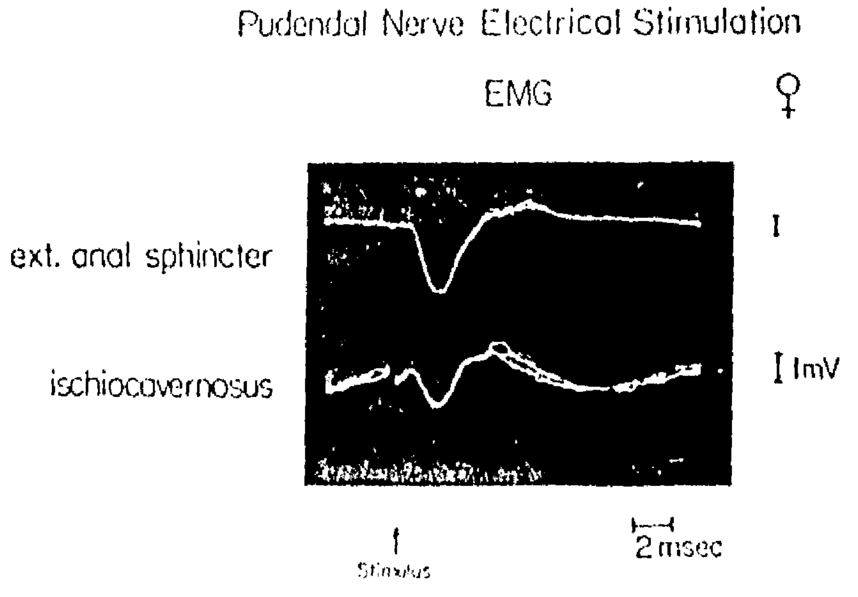


Fig. 5. Electrical stimulation of the pudendal nerve produces contraction of the external anal sphincter and the ischiocavernosus muscles. Contraction of the coccygeus muscle is also produced by this stimulation.

The caudal insertion of the ilio- and pubococcygeus muscles is surrounded by connective tissue that is attached to the peritoneal surface of the vaginal wall. When contraction of these muscles was elicited by electrical stimulation of the pelvic nerve (0.1 ms pulses, 5× threshold to evoke an EMG response), intravaginal pressure, measured by balloon, increased in proportion to the increase in pulse frequency (Fig. 6, upper). Pudendal nerve electrical stimulation was observed to decrease intravaginal pressure (Fig. 6, lower).

Sensory fields of the pelvic nerve branches

Electrical activity recorded from the pelvic nerve

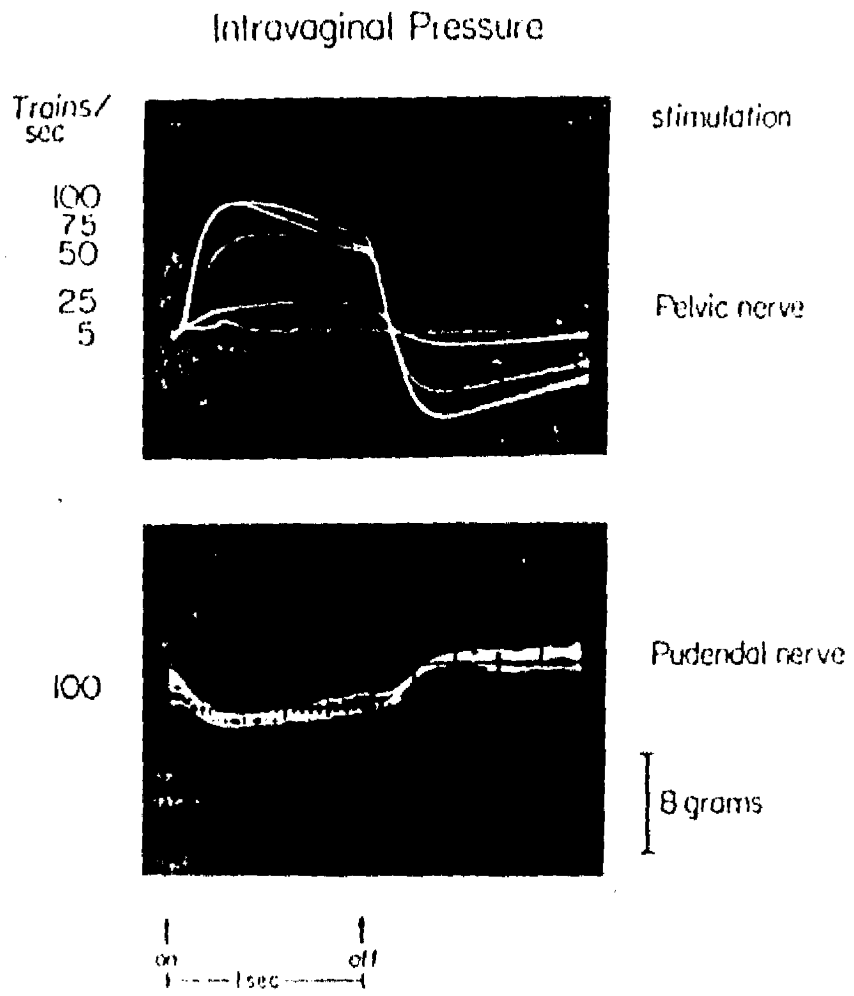
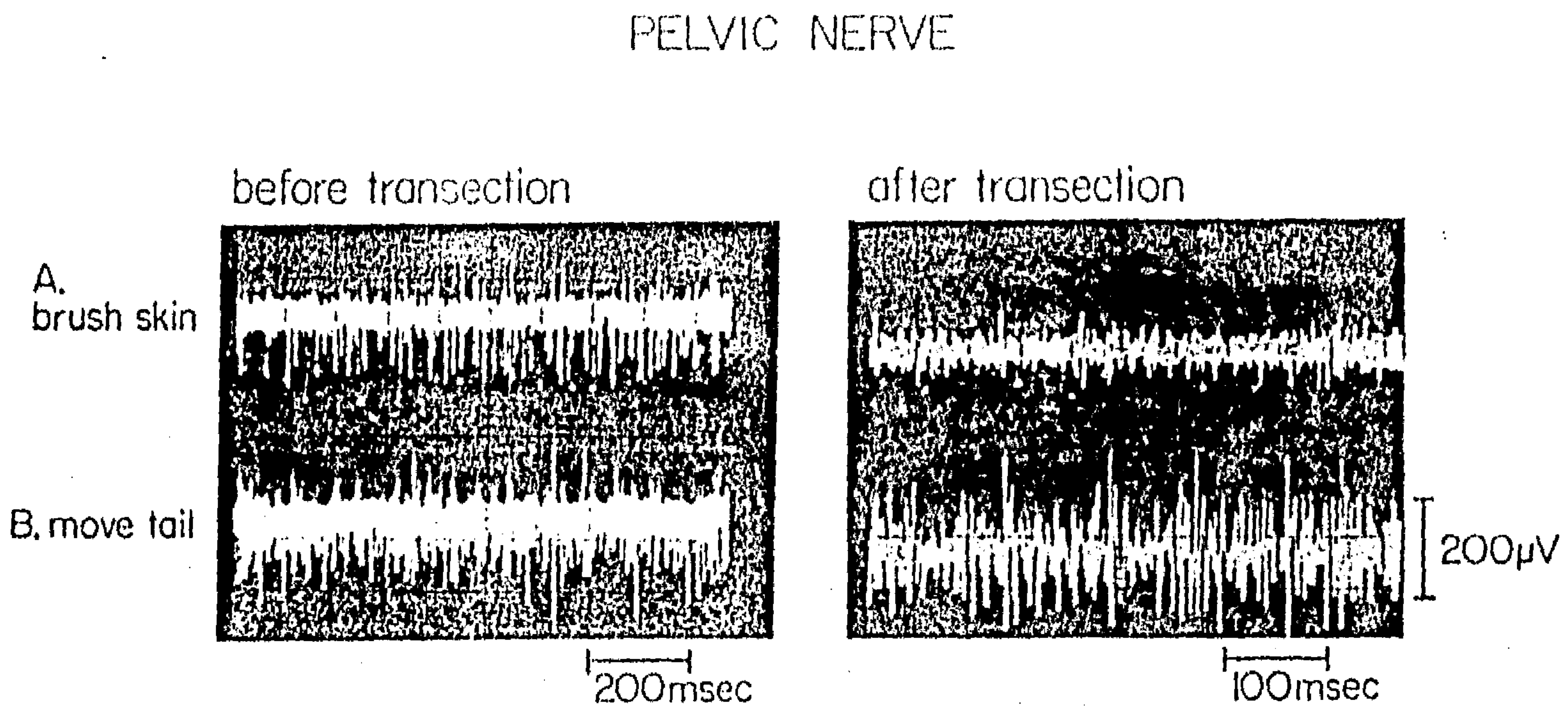


Fig. 6. Electrical stimulation of the pelvic or pudendal nerve produce an increase or decrease, respectively, of intravaginal pressure measured by a balloon plethysmograph.



ig. 7. Recording from the pelvic nerve before and after transection of its viscero-cutaneous branch. The pelvic nerve response to nidline perineal skin stimulation (A, left) is abolished after transection (A, right), although the pelvic nerve response to passive ail displacement (B, left) persists (B, right).

ear its separation from the L_6–S_1 trunk was evoked y both brushing the skin near the midline between he vaginal opening and anus (Fig. 7A, left), and by nanual displacement of the tail to the side contra-ateral to the nerve recording (Fig. 7B, left). When he viscero-cutaneous branch of the pelvic nerve was ut, the nerve activity elicited by the skin stimulation isappeared (Fig. 7A, right), whereas that evoked y tail displacement persisted (Fig. 7B, right). When nly the muscular branch of the pelvic nerve was cut and the viscero-cutaneous branch remained intact, the activity evoked by skin stimulation persisted.

Reflexive activity of the pelvic muscles

Contraction of the ilio- and pubococcygeus muscles was observed during mechanical stimulation of the external genital sensory field of the pudendal and pelvic nerves. In order to further characterize this effect, EMG recordings of the iliococcygeus, pubococcygeus, and flexor caudae brevis were obtained

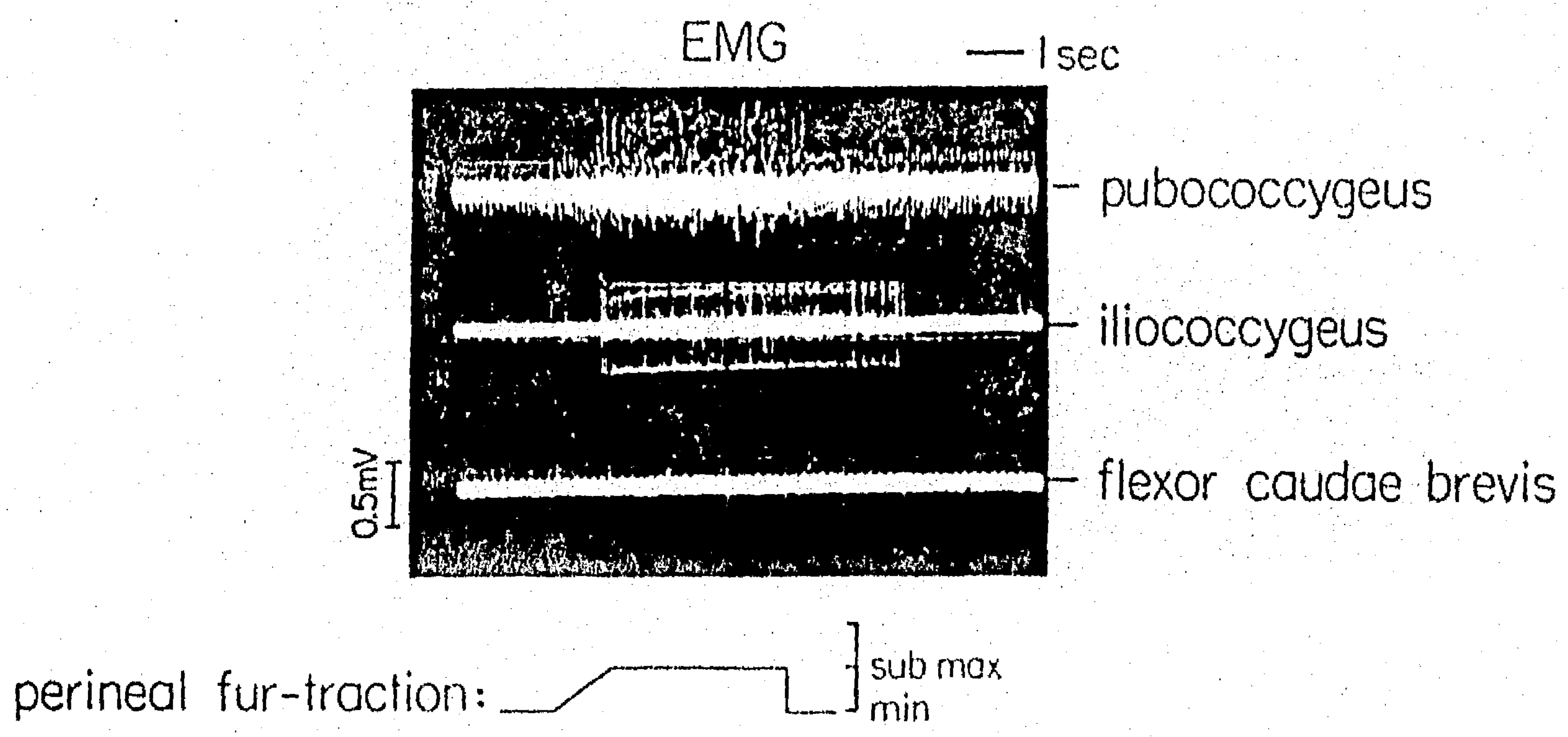


Fig. 8. Clitoral sheath, perineal traction, and/or distal vaginal stimulation activates a reflexive response of the pubococcygeus and iliococcygeus muscles.

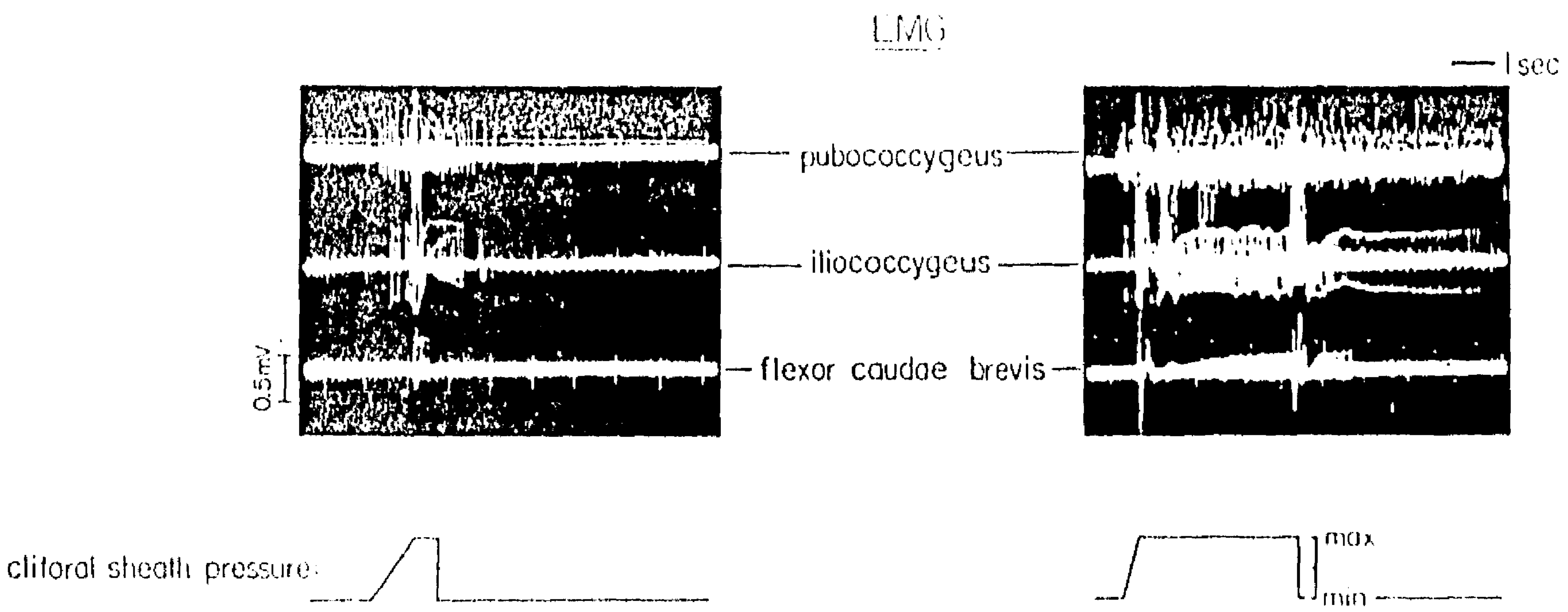


Fig. 9. Pelvic musculature activated reflexively by pressure applied to the clitoral sheath.

during mechanical stimulation of the clitoral sheath, perineal fur, or vagina. Traction applied to the perineal fur evoked reflex muscular activity of pubo- and iliococcygeus muscles, but not of flexor caudae brevis (Fig. 8). Pressure applied to the clitoral sheath provoked EMG activity in the three muscles, particularly in the pubo- and iliococcygeus muscles (Fig. 9). Stimulation of the distal portion of the vagina with a glass probe activated the three muscles (Fig. 10), although not always the flexor caudae brevis (Fig. 11). If the probe touched the cervix, the reflexive muscular activity was immediately abolished (the vaginal wall was still being stimulated by the presence of the probe), and the muscular activity returned upon cessation of the cervical stimulation (Fig. 10). When the probe was removed from the vagina the reflexive muscular activity continued for several seconds (Fig. 11). Since the cervical stimulation was applied manually with a probe and the pubococcygeus muscle is near the vaginal wall, some movement artifacts were frequently present in the recordings (e.g. Fig. 11).

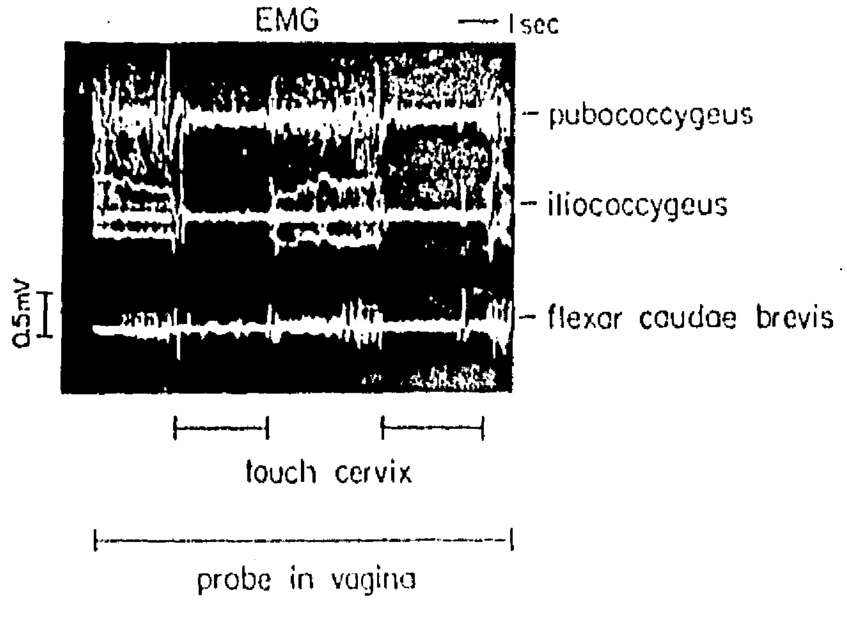
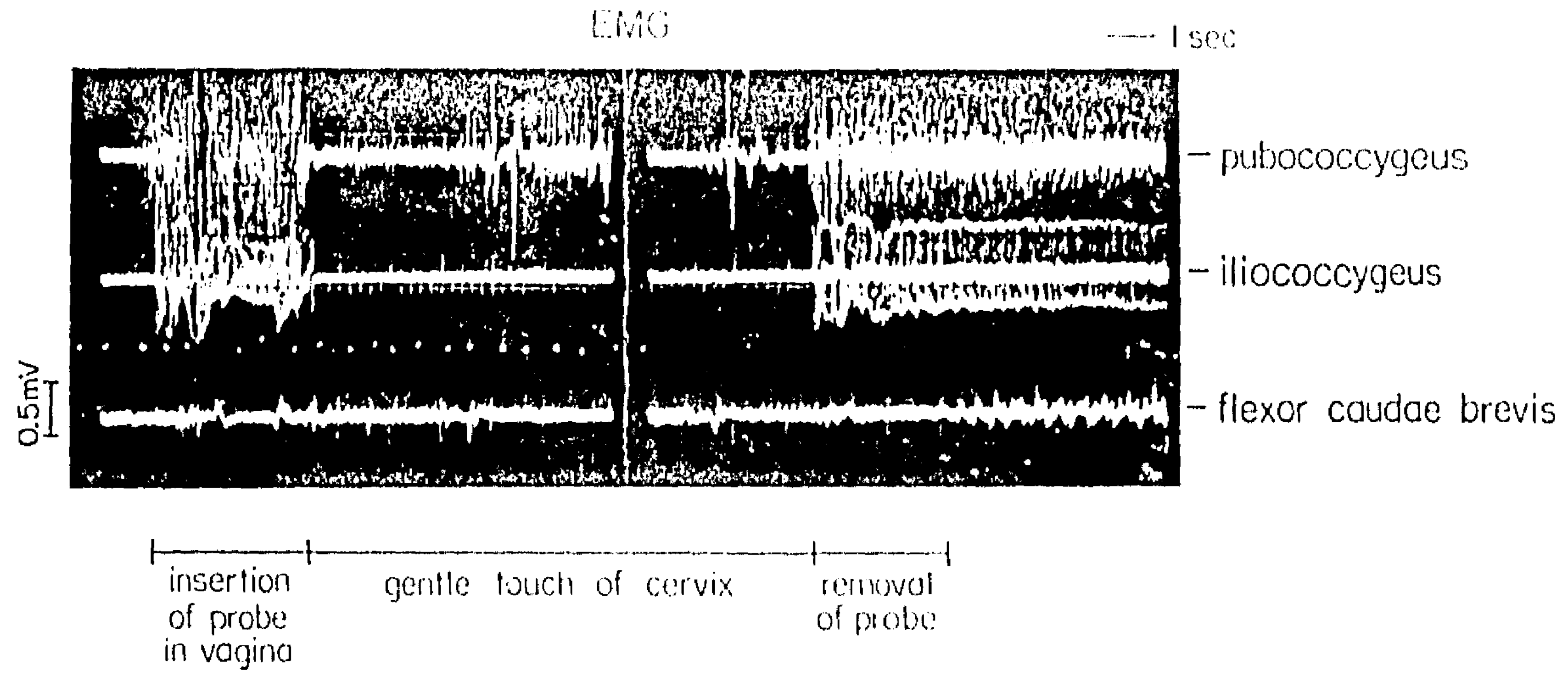


Fig. 10. Gentle mechanical stimulation of the cervix inhibits the activity of pelvic musculature provoked by vaginal stimulation.

DISCUSSION

The pudendal and pelvic nerves are important components of the spinal control of sexual, reproductive and excretory functions. However, there is confusion about the anatomy and nomenclature of these nerves in the rat. The pudendal nerve is most commonly described as originating from L$_6$–S$_1$ spinal cord segments[2,6,19,30]. Confusion exists regarding the origin of the pelvic nerve; Greene[12] does not mention it, others claim that it is a branch of the pudendal nerve[19] that it emerges from S$_1$ and S$_2$[37] or that it originates from the L$_6$–S$_1$ trunk[2,30]. The present anatomical observations support previous observations[2,30] that the pudendal and pelvic nerves travel primarily in the L$_6$–S$_1$ trunk. However, this trunk has two variations (see Fig. 1), which can help to account for individual variability in shape and location of the receptive field recorded from some lumbar dorsal roots of the rat[26].

The pelvic nerve, after its separation from the trunk, travels caudally and bifurcates. One branch



g. 11. Cervical stimulation inhibits the reflexive response of the pubococcygeus and iliococcygeus muscles to vaginal stimulation. e response persists after withdrawal of the vaginal stimulation. Notice some movement artifacts provoked by the probe which s applied manually.

nsists of 3 bundles traveling to the midline of the dy, the 'viscero-cutaneous' branch. The other, ore compact 'muscular' branch, travels caudally d then bifurcates. It has been suggested[30] that this uscular branch innervates only the iliococcygeus uscle; however, based on electromyographic recordings, we find that one of its branches innervates e iliococcygeus muscle and the other branch nervates the pubococcygeus muscle. Recently it s suggested that one of the pelvic nerve branches rns caudally and sends branches into muscle and, parently terminates in perineal skin'[35]. Present sults indicate that the pelvic nerve branch that rries both vaginal and skin afferents[35] corresponds the viscero-cutaneous branch.

In summary, the viscero-cutaneous branch of the lvic nerve receives sensory input from the perigetal skin as well as viscera[35] and it is also one of the ferent pathways from the spinal cord to the pelvic nglia[36]. The muscular branch of the pelvic nerve ovides efferent innervation of the ilio- and pubococcygeus muscles and conveys sensory activity from em.

In the female rat, the pudendal nerve distributes series of delicate fibers to the coccygeus muscle[37] d the extremely fine ischiocavernosus muscle[30]. In is study the electrical stimulation of the pudendal rve caudal to its separation from the pelvic nerve cited contractions of the coccygeus, external anal sphincter and ischiocavernosus muscles that were corroborated by the EMG activity of these muscles.

Based on anatomical studies, it has been claimed that the ischiocavernosus, bulbocavernosus and levator ani muscles are completely involuted in adult female rats[7,14,43]. The lumbar spinal cord motoneurons that innervate these muscles are smaller and fewer in female than in male rats[5,18,30]. Despite this evidence, the ischiocavernosus muscle has been identified and observed to contract upon electrical stimulation of the pudendal nerve in adult female rats[30]. The present study in adult female rats confirms and extends the latter finding, showing that electrical stimulation of pudendal nerve stimulates EMG activity in the ischiocavernosus muscle.

The movements of the orifice and wall of the vagina and the intravaginal pressure changes observed during electrical stimulation of the two nerves suggest some specific functions of these nerves in addition to those postulated in copulation and parturition[35]. Perhaps during copulation the reflex activation of both nerves provokes closure of the anal orifice and opening of the vaginal orifice, thereby facilitating penile intromission. The reflexive movements of the wall of the vagina and increment and decrement of the intravaginal pressure may facilitate ejaculation. The contraction of ilio-, pubo- and coccygeus muscles may produce a rigidity and consequently fixation of the perineal wall of the female rat, thereby facilitating the intromission. When ejaculation occurs, the seminal plug may stimulate the cervical receptors, provoking the inhibition of the muscular contractions and closing the vaginal orifice.

Many of the physiological and behavioral adaptations serving reproduction in mammals are initiated by tactile stimulation, especially in the genital region[1,6,13,19,42]. The motoneuron cell populations of the lumbosacral spinal cord innervate visceral and skeletal muscles which control voiding, defecation and sexual function[27,32,33]. The functional interaction between these cell groups has received minimal attention[11]. The present results, showing reflexive excitatory and inhibitory EMG activity in the ilio- and pubococcygeus muscles during mechanical stimulation of the external and internal genitalia, indicate an interaction between pudendal and pelvic nerve function. The inhibitory effect of cervical stimulation observed in the present study is consistent with other motor inhibitory[21] and analgesic effects[22] reported for cervical mechanostimulation (see ref. 23 for review). The pelvic-pudendal interactions described electrophysiologically in the present study may provide a useful model for studying visceral-somatic sensorimotor relationships.

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Visceral and postural reflexes evoked by genital stimulation in urethane-anesthetized female rats

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The present study describes several muscular reflexes produced by genital stimulation, the nerves that subserve them, and the visceral and postural effects induced by these reflexes. Electrical stimulation of the iliococcygeus (ic) and pubococcygeus (pc) (striated) muscles produced movement of the vaginal orifice and wall, membranous urethra, tail and pelvis. Electrical stimulation of the psoas major (pm) or iliacus (i) (striated) muscles produced movements of the lumbar vertebrae and extension of the ipsilateral hindlimb. Sensory mechanostimulation elicited responses of these muscles as follows: stimulation of the perineal skin, clitoral sheath or distal vagina produced reflex contraction of the ic and pc muscles. Stimulation of the cervix produced reflex contraction of the pm and i muscles and also blocked the above reflex contraction of the ic and pc muscles. Both the cervical stimulation-induced blockage of the ic and pc reflex response, and the cervical stimulation-induced activation of pm and i muscles was prevented by bilateral transection of the viscerocutaneous branch of the pelvic nerve. Based on the above observations, it is proposed that stimulation of the vaginal surface of the cervix resulting from penile intromission and/or seminal plug deposition during mating behavior in the rat may reflexively activate pm and i, thereby contributing to the hindleg postural rigidity and lordotic dorsiflexion that are characteristic of the normal mating posture in female rats.

INTRODUCTION

While the pelvic musculature undoubtedly plays a significant role in copulatory and parturitional processes, there is little experimental data regarding its participation in these processes and the related genital reflexes.

The striated ilio- (ic) and pubococcygeus (pc) muscles of the rat, which are innervated by motoneurons localized in several nuclei of lamina IX in the lumbosacral transition zone of the spinal cord[17,24], are activated reflexively by mechanostimulation of the clitoral sheath, perineum or lower vagina[20]. Gentle cervical mechanostimulation blocks this reflexive activation[20]. Electrical stimulation of the 'motor' branch of the pelvic nerve, which innervates these muscles, increases intravaginal pressure[20]. Although these muscles have a skeletal origin and insertion[3,8,9] they are also attached to the intraabdominal surface of the vaginal wall via connective tissue[20]. This anatomical feature could account for changes in vaginal pressure that are produced by contraction of these skeletal muscles.

Mechanical stimulation of the upper vagina and cervix in urethane-anesthetized, periparturient rats evokes the 'fetus expulsion reflex' by increasing intraabdominal pressure through reflex contraction of unspecified abdominal musculature and the diaphragm[11]. Thus, it seems that cervical stimulation evokes in the abdominal muscles an effect (activating) different from that obtained in ic-pc muscles (blocking of reflexive activation)[20]. The psoas major (pm) and iliacus (i) muscles are abdominal muscles that could participate in genital reflexes.

Therefore, in the present study we analyzed the following processes: nature of the ic-pc and pm-i muscle reflexive activity evoked by genital stimulation, the primary afferent pathways mediating the above mentioned muscle response, and the visceral and somatic effects of contraction of these muscles.

MATERIALS AND METHODS

Urethane anesthetized (1.6 g/kg in aqueous 20% solution by weight i.p.) Sprague–Dawley rats (250–300 g) were used. The study utilized 20 intact virgin females. During the experiments, supplemental doses of urethane were administered as necessary and

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at the end of the experiment the animals were euthanized with an i.v. overdose of urethane. The animals were maintained in a supine position for the duration of the experiment. After a midline ventral abdominal wall incision, the viscera were carefully displaced from the ic-pc and pm-i muscles. All viscera and exposed tissue were kept moist with warm saline solution. Pairs of stainless steel wires (0.1 mm diameter) were inserted into the muscles and prepared for gross electromyographic (EMG) recordings or electrical stimulation. The EMG signals were amplified by GRASS 7P3 wideband AC preamplifiers, displayed on a TEKTRONIX 564 storage CRO and photographed. Since the EMG was composed of potentials of different amplitude (see Fig. 1), our analysis was based only on the presence or the absence of activity. Muscles were electrically stimulated with 0.1 ms square wave pulses at 0.5–100 Hz and variable intensities (up to 30 V) that were modulated by the contraction response. Pulses were delivered through a GRASS SIU-1 stimulus isolation unit activated by a GRASS S48 stimulator. Care was taken to avoid displacement of the electrodes.

Dissections were performed on 3 rats in order to corroborate the origin, course, and insertion of the ic-pc and pm-i muscles[3,8,9]. Detailed microscopic analysis of the membranous urethra and surrounding tissue was performed in two rats with the use of a Zeiss dissecting microscope. It was corroborated that the ic-pc muscles have their origin in the innominate bone of the pelvis and their insertion in caudal vertebrae Ca_1-Ca_6 (see Fig. 8). Near the ventromedial surface of the pubic bone, fibers from both muscles spread out from the pelvic cavity to insert along the diapophyses of the proximal tail via fleshy insertions or by means of small fascia strands[3]. It was also observed that before and near their caudal insertion the two muscles are surrounded by abundant connective tissue that is attached to the peritoneal surface of the vaginal wall. Fibers that comprise the medial border of the pc muscle are also attached through connective tissue to the peritoneal surface of the membranous urethra.

The pm-i muscles are two of the 4 striated muscles that form the dorsal abdominal wall. In agreement with previous observations[8,9], the origin of the pm muscle is on the bodies and ventral surface of the transverse processes of vertebrae L_2-L_6. The origin of the i muscle is on the transverse processes of the vertebrae L_5-L_6 and part of the vertebral surface of the ilium. The insertion of these muscles is on the lesser trochanter of the femur via a long tendon (see Fig. 8).

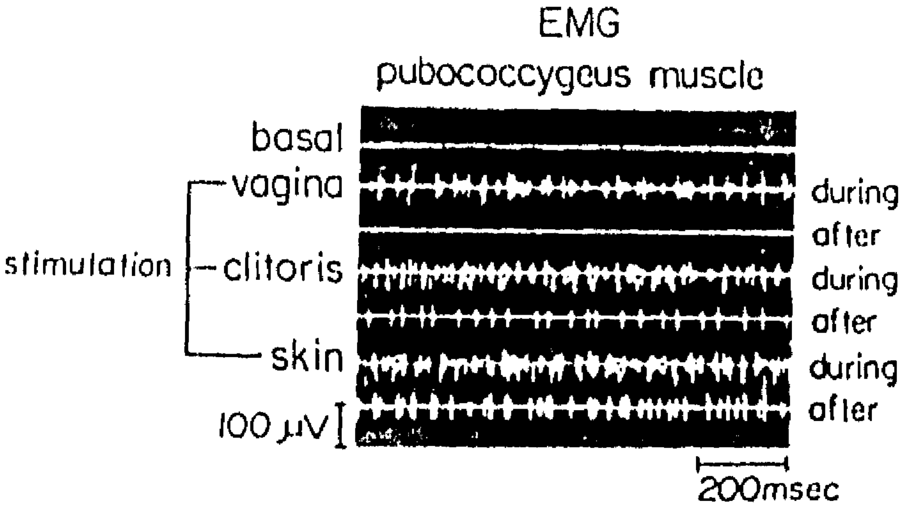


Fig. 1. Electromyograph (EMG) of the pubococcygeus (pc) muscle during genital stimulation. There was no spontaneous EMG activity in the pc muscle. One second of probing the distal vagina evoked EMG activity that ceased immediately after the probe was withdrawn (in all cases the 'during' trace was continuous with the 'after' trace). One second of traction applied to either the clitoral sheath or the perineal skin evoked an EMG response that persisted after cessation of the stimulation (i.e. afterdischarge).

RESULTS

Visceral and somatic effects of electrical stimulation of the ic-pc and pm-i muscles

Direct electrical stimulation of the ic or pc muscles produced ipsilateral movement of the orifice and wall of the vagina. Contraction of these muscles moved the tail towards the midline when it was placed contralateral to the stimulated side. No tail movement was elicited by electrical stimulation of the ic or pc muscles when the initial position of the tail was at the midline. Electrical stimulation of the ic or pc muscles elicited pelvic thrusting following the stimulation frequency. The vigor of pelvic movements increased in direct relation to the pulse frequency. Stimulation of the pc muscle also produced movement of the membranous urethra.

Unilateral electrical stimulation of the pm or i muscles produced movement of the lumbar vertebrae and extension of the ipsilateral hindlimb. This effect was most readily observed when the pelvis was held in a fixed position. In all the above observations, the rats were supine.

Effects of stimulation of genital areas

Mechanostimulation of the perineal skin, clitoral sheath or lower vagina elicited reflexive EMG activity in the ic and pc muscles. Afterdischarge (i.e. EMG activity persisting beyond the cessation of sensory stimulation) occurred in response to stimulation of the perineum or clitoral sheath, but not distal vagina (Fig. 1) and was related to the stimulus duration (Fig. 2).

Whereas the above stimuli had no observable effect on the EMG of the pm and i muscles, when the intravaginal probe touched the cervix, these muscles became strongly activated (Fig. 3). This stimulus did not induce afterdischarge. However, when the cervical stimulation was repeated several times in succession (stimulation 'on' 2 s, 'off' 2 s) a post-stimulation afterdischarge was

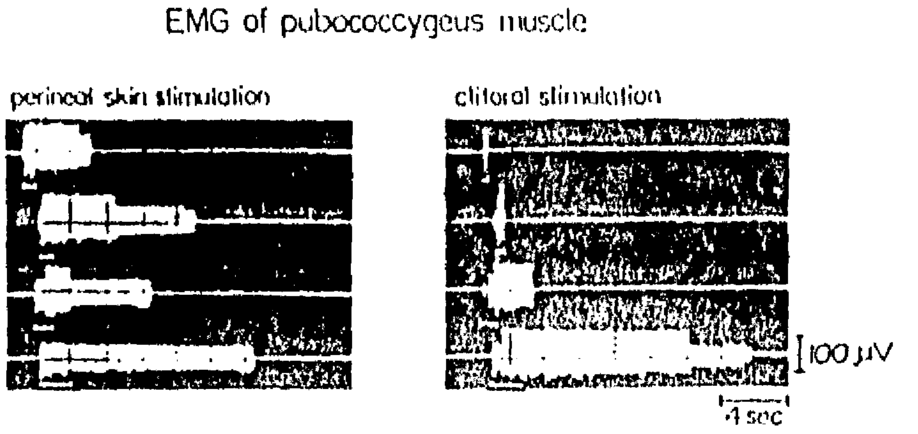


Fig. 2. Traction applied to the perineal skin or clitoral sheath evoked EMG activity of the pc muscle that persisted as a post-stimulation afterdischarge. The duration of the afterdischarge was positively related to the duration of the stimulation. Stimulus duration is indicated by the bars below the traces.

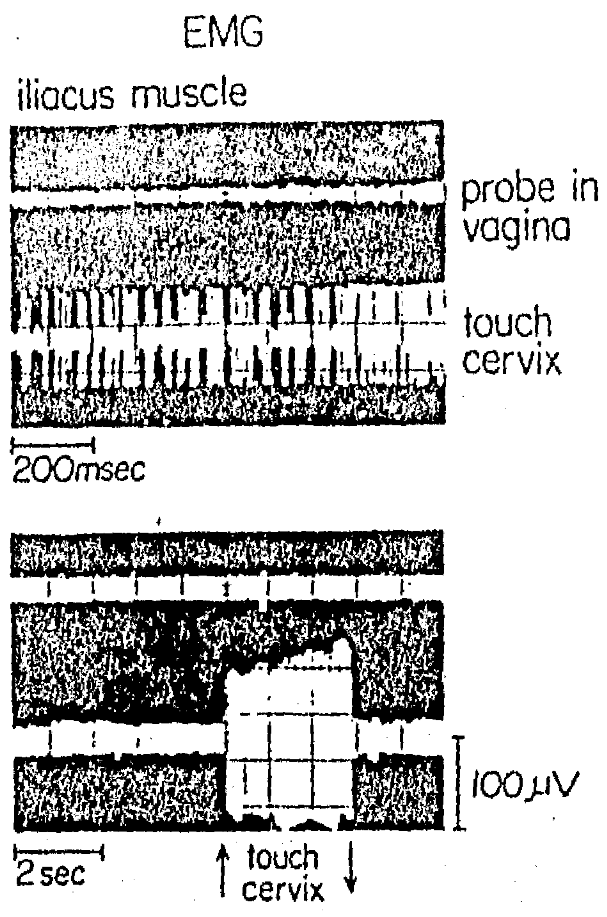


Fig. 3. EMG of the iliacus (i) muscle during vaginal and cervical stimulation (traces shown in immediate succession). Although mechanical probing of the distal vagina did not affect the EMG activity of the i muscle, an EMG response was observed when the probe touched the cervix. Upper and lower trace of each panel: before and during cervical touch, respectively. In the bottom trace, note the absence of afterdischarge.

induced in the pm and i muscles (Fig. 4). In none of these 4 muscles in which EMG was recorded was any 'spontaneous' EMG activity observed.

Nerves mediating the ic-pc and pm-i muscle reflexes

To ascertain the role of the innervation of the external genitalia[14-16] in the reflex contraction of the ic-pc and pm-i muscles, bilateral transections of the viscerocutaneous branch of the pelvic[3,20], the pudendal, or the genitofemoral nerves was performed (Fig. 5).

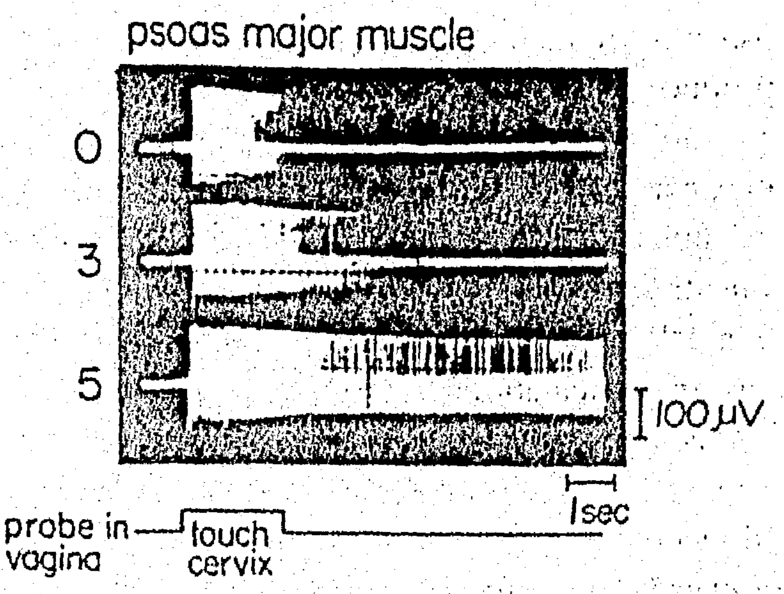


Fig. 4. Cervical probing produced a strong EMG activation of the pm muscle. The first cervical stimulation (at 0) evoked EMG activity of the pm muscle without an afterdischarge. When the cervical stimulation was repeated several times in succession (stimulation 'on' 2 s, 'off' 2 s), a post-stimulation afterdischarge was observed. The afterdischarge duration was positively related to the number of times (3 or 5) that the cervix was stimulated.

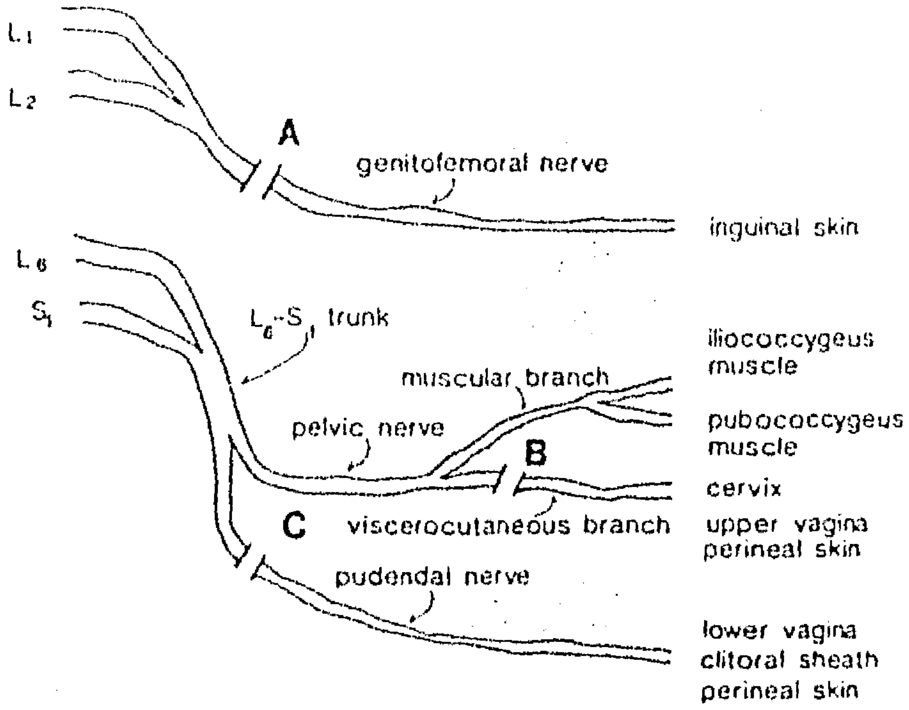


Fig. 5. Schematic diagram of the nerves and branches that were transected in the present study. A: the genitofemoral nerves were transected just caudal to the aortal bifurcation. B: the viscerocutaneous branch of the pelvic nerve was transected at the level of the medial side of the internal iliac vein. C: the pudendal nerve was transected 5 mm caudal to the bifurcation of the L$_6$-S$_1$ trunk. The afferent relationships of the 3 nerves[1,4,14-16,20-22] are listed.

Fig. 6, which is representative of 3 animals, shows that cervical stimulation activated the i muscle and blocked

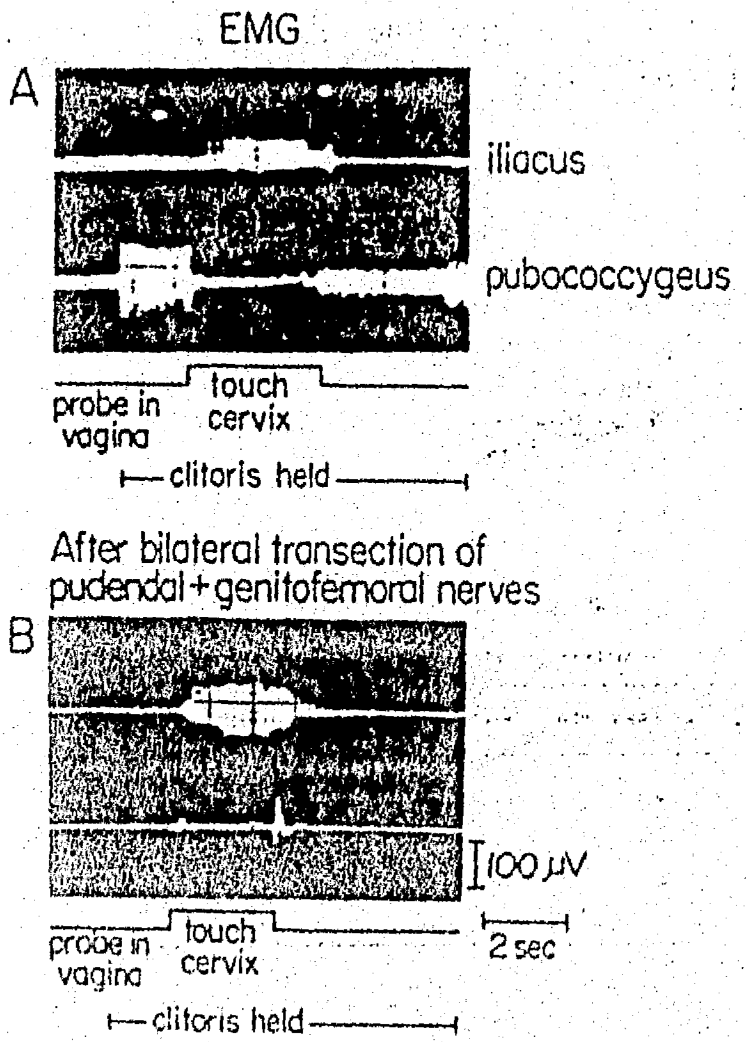


Fig. 6. Bilateral transection of pudendal and genitofemoral nerves differentially blocked pc reflex contraction induced by clitoral stimulation but not i muscle contraction induced by cervical stimulation.

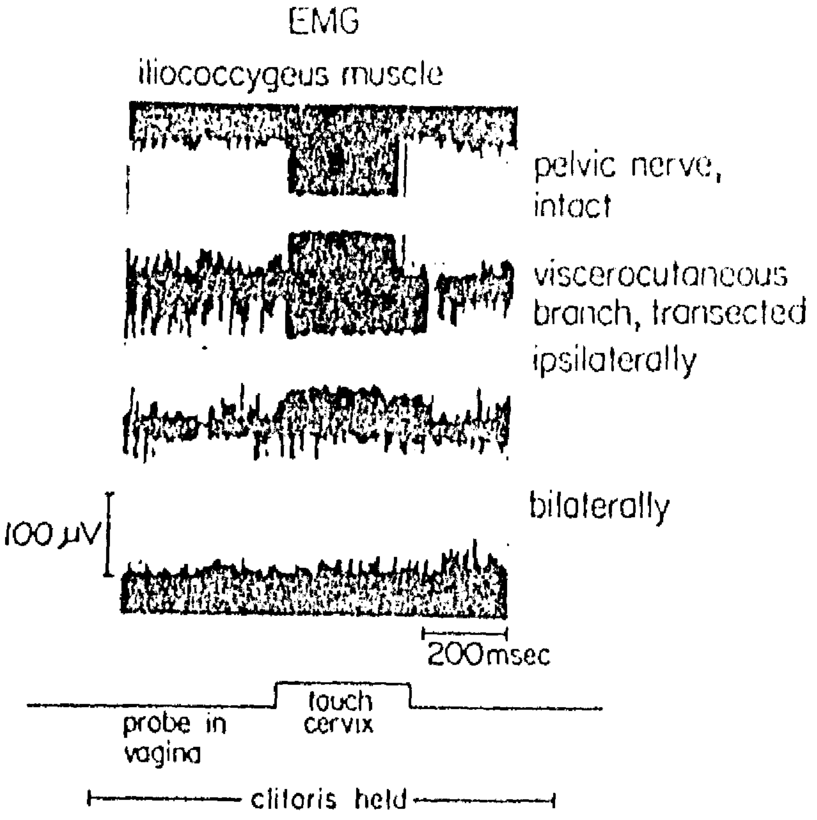
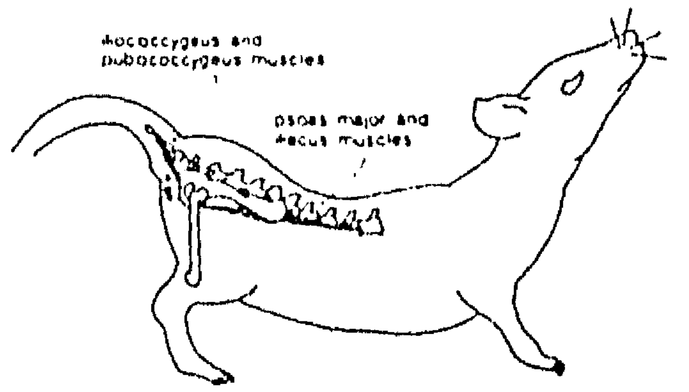


Fig. 7. Bilateral transection of the viscerocutaneous branch of pelvic nerve completely abolished the inhibitory effect of cervical stimulation on ic muscle contraction induced by clitoral traction. Unilateral transection exerted only a partial effect.

the activation of the pc muscle that was evoked by clitoral stimulation. Combined bilateral pudendal and genitofemoral transection abolished the pc muscle activation that was evoked by clitoral stimulation, but did not affect the i muscle activation evoked by cervical stimulation. These effects were restricted to the time of stimulus application. Bilateral transection of either the pudendal or the genitofemoral nerves (but not both) did not block the pc muscle activation that was produced by clitoral stimulation (not shown).



SUMMARY OF REFLEXIVE PATHWAYS

SENSORY FIELD	AFFERENT NERVE	EFFECTOR (MUSCLE)	MOTOR RESPONSE
cervix	pelvic	psoas major iliacus	• lordotic dorsiflexion • hindleg extension • "bearing down" movements
cervix	pelvic	iliococcygeus pubococcygeus	"bottleneck" inhibition
• lower vagina • perineal skin • clitoral sheath	pudendal genitofemoral*	iliococcygeus pubococcygeus	• tail movement • pelvic thrusting • vaginal movement • urethral movement

(*) Clitoral sheath traction can activate genitofemoral nerve afferents via indirect movement of the skin of the caudal abdomen.

Fig. 8. Summarizes the reflexive pathways and represents the relationships of the 4 muscles studied.

As shown in Fig. 7, bilateral transection of the viscerocutaneous branch of the pelvic nerve abolished the i muscle response that was evoked by cervical stimulation, whereas unilateral neurectomy produced only a partial effect. The bilateral transection also abolished the blocking effect of cervical stimulation on the ic and pc muscle response. These effects were observed in each of 4 animals.

DISCUSSION

Fig. 8 summarizes the possible reflexive pathways involved in the present study and schematizes the origin and insertion of the 4 muscles studied.

The present findings demonstrate differences between the EMG of the pelvic floor (inhibited) and the hindlimb (activated) in response to the same cervical stimulus. We have shown that cervical stimulation, which also evokes contraction of other abdominal and diaphragmatic muscles[10,11], elicits reflexive contractions of the 'postural' pm and i muscles but abolishes the reflexive contractions of the ic and pc muscles produced by perineal stimulation. Therefore, cervical stimulation probably produces excitatory effects upon motoneurons innervating the pm, i, and other abdominal and diaphragm muscles, and inhibits those motoneurons involved in the reflex activation of the ic and pc muscles.

Repeated vaginal-cervical stimulation elicited sensitization of the reflexive afterdischarge of the pm and i muscles (Fig. 4). This effect is in accord with the study by Berkley et al.[1] that clearly shows the sensitization of pelvic nerve afferents with repeated applications of cervical pressure.

The present findings suggest that the pm and i muscle reflex responses to cervical stimulation are involved in the lordosis posture of female copulation. Cervical stimulation produces hindleg extension and immobility[12,13]. Our results indicate that hindleg extension is due in part to the reflex contraction of the pm and i muscles induced by cervical stimulation. Therefore, it appears likely that the prolongation and exaggeration of the lordotic posture response[21-23] observed in female rats following intromissions and ejaculations[6,7,13] or seminal plug deposition[18,19,25] is mediated in part through the responses of the pm-i muscles to cervical stimulation. These effects also could facilitate withdrawal of the penis from the vagina after ejaculation, thereby helping to avoid dislodgement of the copulatory plug[2,19,25]. Transection of the pelvic nerve, which through its cervical innervation is involved in the pm and i response, also shortens the duration of lordosis and immobility[6].

It has been suggested that in peripartutient rats, vaginal distension produced by the fetus facilitates delivery by increasing intraabdominal pressure through contraction of the abdominal muscles and diaphragm[11]. It is possible that pm and i response is also involved in the process of parturition. These muscles, whose origin is on the bodies and transverse processes of vertebrae L_2–L_6 (see Fig. 8), participate in the 'bearing down' movements observed during expulsion by producing rigid hindleg extension and by pulling down on the lumbar vertebrae. The findings that bilateral, but not unilateral, neurectomy of the pelvic nerve prolongs the delivery process[10,11] and that a similar surgical procedure blocks pm and i reflex contractions induced by cervical stimulation (present study) supports the idea that the pm and i muscles normally participate in parturition.

Bilateral neurectomy of the viscerocutaneous branch of the pelvic nerve eliminates the blocking effect of cervical stimulation on the reflexive contractions of the ic-pc. Contraction of these muscles may prevent the passage of the fetus through the birth canal since their contraction increases intravaginal pressure[20], thus creating a 'bottleneck'. The 'bottleneck' effect may be prevented by the inhibition exerted on these muscles by the head of the fetus stimulating the cervix. This normal inhibitory effect would dilate the vaginal canal. It appears likely that pelvic nerve transection also impedes parturition by preventing the occurrence of this cervical stimulation-induced inhibitory effect on the ic and pc muscles.

The investment of connective tissue both in the ic-pc muscles and the peritoneal surface of the vaginal wall provides a structural arrangement that could account for the increase of intravaginal pressure that occurs during contraction of the ic-pc muscles[20]. In the present study, the connective tissue invested in the pc muscle was also observed to invest in the peritoneal surface of the membranous urethra. Recently it was shown in female rats that gentle rhythmic mechanical stimulation of the urethra elicits the 'coitus reflex', which consists of regular, rhythmic bursts of activity in the pudendal nerve motor branch and the cavernous nerve, and rhythmic movements of the vagina, leading to increases in vaginal pressure[5]. It is possible that clitoral sheath traction could to some extent stimulate also the urethral bulb, thereby eliciting ic-pc muscular reflex contraction. It is tempting to speculate that as a consequence of the connective tissue junction between these skeletal muscles and the vagina and urethra, sensory receptors in these muscles and/or connective tissue could be indirectly stimulated by vaginal and/or urethral distention or alternatively, contractions of these muscles could exert traction on the urethral bulb. Consequent afferent activity from, and/or reflex contraction of, these skeletal muscles could contribute to copulatory and/or micturition reflexes.

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Participation of Pelvic Nerve Branches in Male Rat Copulatory Behavior

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LUCIO, R. A., J. MANZO, M. MARTÍNEZ-GÓMEZ, B. D. SACHS AND P. PACHECO. *Participation of pelvic nerve branches in male rat copulatory behavior.* PHYSIOL BEHAV 55(2) 241–246, 1994.—The role of the pelvic nerve branches in the mediation of copulatory behavior was investigated. The somatomotor or the viscerocutaneous branch of the pelvic nerve was bilaterally sectioned in sexually experienced male rats. Somatomotor branch surgery had no detectable effect. Viscerocutaneous branch transection altered copulatory parameters that reflect impairments in penile erection and seminal plug emission. The altered behavioral parameters approached or reached presurgical and sham values 21 days after transection, indicating that the damage to erectile and ejaculatory function was transient. It is suggested that animals with viscerocutaneous branch transection recover copulatory efficiency through a compensatory plastic mechanism, possibly involving the hypogastric nerve.

Copulatory behavior Penile erection Ejaculation Seminal plug Pelvic nerve Hypogastric nerve Plasticity Nerve injury

IN rats the pelvic nerve (PvN) intermingles with the hypogastric nerve in the major pelvic ganglion, forming the pelvic plexus (15,26). The PvN controls pelvic organs related to the reflex functions of micturition and defecation (32) through both sympathetic and parasympathetic autonomic fibers (14). In male (22), as in female rats (24), the PvN has two branches, the viscerocutaneous (VC) and the somatomotor (SM). In males, the VC is composed of axons that carry efferent innervation to the major pelvic ganglion (14) and afferent innervation from the urinary bladder (21,22), and from scrotal and perianal skin (22). As in females (24), the SM branch in males (22) has sensory and motor axons that innervate the iliococcygeus and pubococcygeus muscles.

On the basis of physiological and behavioral studies (19,25), the PvN is well known to be involved in female mating behavior (and other reproductive functions), and physiological studies have implicated the PvN in erectile function of rats (6,27) as well as humans (7). However, only one study has explored PvN participation in male rat copulatory behavior (20), and the authors came to the unexpected conclusion that bilateral PvN transection does not interfere with the mechanism for the induction or maintenance of erection or with the expression of normal mating behavior. Considering the structural and functional complexity of the PvN and its previous implication in the process of erection, it is difficult to understand why its transection did not alter mating behavior (20).

In the present study, we discovered major, but transient, impairments in copulatory and ejaculatory function in rats with bilateral transection of the PvN VC branches. The copulatory deficits were attributable to impaired erectile function. Transection of the SM branch was without apparent effect.

METHOD

Subjects

Sexually experienced male Wistar rats, weighing 300–350 g, were randomly assigned to five groups: a) PvN SM branch bilaterally sectioned (Exp 1, $n = 13$); b) Sham SM (Sh 1, $n = 5$) animals; c) PvN VC branch bilaterally sectioned (Exp 2, $n = 17$); d) Sham VC (Sh 2, $n = 6$) rats; and e) males used to dislodge seminal plug from vagina (see the following paragraphs).

Rats were housed in plastic cages (50 × 30 × 20 cm) containing wood chip bedding, commercial pelleted rodent chow, and water ad lib. Cages were placed in a room maintained at 24°C, and LD 12:12 light schedule, lights on at 2200 h.

Surgery

Nerve transections were carried out under sodium pentobarbital anesthesia (26 mg/kg IP). PvN branches were sectioned

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as described elsewhere (24). Briefly, after an extensive ventral abdominal incision, branches were located where they cross the internal iliac vessels. The VC travels more laterally than the SM branch. To avoid regeneration, a 5 mm portion of the nerve was extracted. During sham surgery the branch was dissected and hooked but not cut. Because VC branch bilateral transection interferes with micturition, the bladder was manually expressed three times daily after surgery. This procedure, plus careful surgery that prevented bladder wall bleeding, allowed all animals to remain healthy. At the end of the study the animals were deeply anesthetized, and the branch transections were confirmed before the rats were sacrificed with an overdose of pentobarbital. The postmortem examination of all the animals with nerve transections revealed no evidence of regeneration.

Mating Tests

Two consecutive ejaculatory series (I, II) of a mating test were conducted on days 11, 8, 5, and 2 before surgery and days 3, 6, 9, 12, 15, 18, and 21 after surgery. These tests were conducted during the last third of the dark period. After a 5 min adaptation in a Plexiglas cylinder arena (50 cm high × 50 cm diameter), a receptive ovariectomized rat (injected with 10 μg estradiol benzoate and 2 mg progesterone, 48 and 4 h before test, respectively) was placed with the male. At this moment the first ejaculatory series began.

If an intromission pattern occurred, testing continued for 30 min or until the ejaculatory pattern appeared. Without an intromission pattern, the test was stopped after 15 min. When an ejaculatory pattern was observed, the female was immediately shifted to another arena. This arena, which had a black floor with no bedding material, was already occupied by a male used to dislodge the seminal plug, if any, from the vagina. Plugs are generally dislodged within three to six intromissions (23). To prevent ejaculation, males used for this procedure were limited to four intromissions, and females were introduced to a second male if it was necessary. If no plug was dislodged within eight intromissions, then it was assumed that none was present. Plugs obtained were immediately weighed. Five minutes after the test male showed the ejaculatory pattern, the female was returned to the first arena, and the second ejaculatory series test was begun. The same criteria for test termination were used for the second ejaculatory series.

Parameters scored during mating were series duration (total time of test), mount latency, total number of mounts (NM), intromission latency, total number of intromissions (NI), ejaculatory pattern latency, and seminal plug weight (2). From these measures we calculated number of mounts per minute, number of intromissions per minute, intromission ratio [NI/(NM + NI)], percentage of males showing ejaculatory patterns, and percentage of males with seminal plugs.

Statistics

Results that are presented as means ± SEM, were analyzed with analyses of variance (ANOVA) for repeated measures, followed by the Tukey Test for significant differences between sham and experimental males. These statistics reflect only those males that showed ejaculatory patterns (see Fig. 6). Data presented as percentages (see Fig. 6) were analyzed with the Fisher exact probability test, and compared sham vs. experimental males. Significant changes are shown with asterisks: *$p < 0.05$ and **$p < 0.01$.

RESULTS

During presurgery tests in both series, 100% of males (n = 41) displayed ejaculatory patterns. After surgery, animals with bilaterally transected PvN SM branch (Exp 1) presented no parameters significantly different from those of sham (Sh 1) animals (data not shown).

After surgery, animals with bilateral sectioning of the PvN VC branch (Exp 2) showed changes in most measures of copulation. Thus, as compared with sham (Sh 2) animals, the total number of mounts (Fig. 1) of both ejaculatory series were significantly increased by surgery [series I: $F(1, 21) = 8.49$, $p < 0.01$; series II: $F(1, 21) = 6.88$, $p < 0.05$]. Also, this parameter showed significant change over the course of postsurgery testing [series I: $F(6, 126) = 7.89$, $p < 0.005$; series II: $F(6, 126) = 8.67$, $p < 0.005$]. The surgery–test interaction was also significant [series I: $F(6, 126) = 2.42$, $p < 0.05$; series II: $F(6, 126) = 2.89$, $p < 0.01$]. However, post hoc (Tukey test) analyses for both ejaculatory series showed a significant surgery effect only on day 3 postsurgery (Fig. 1).

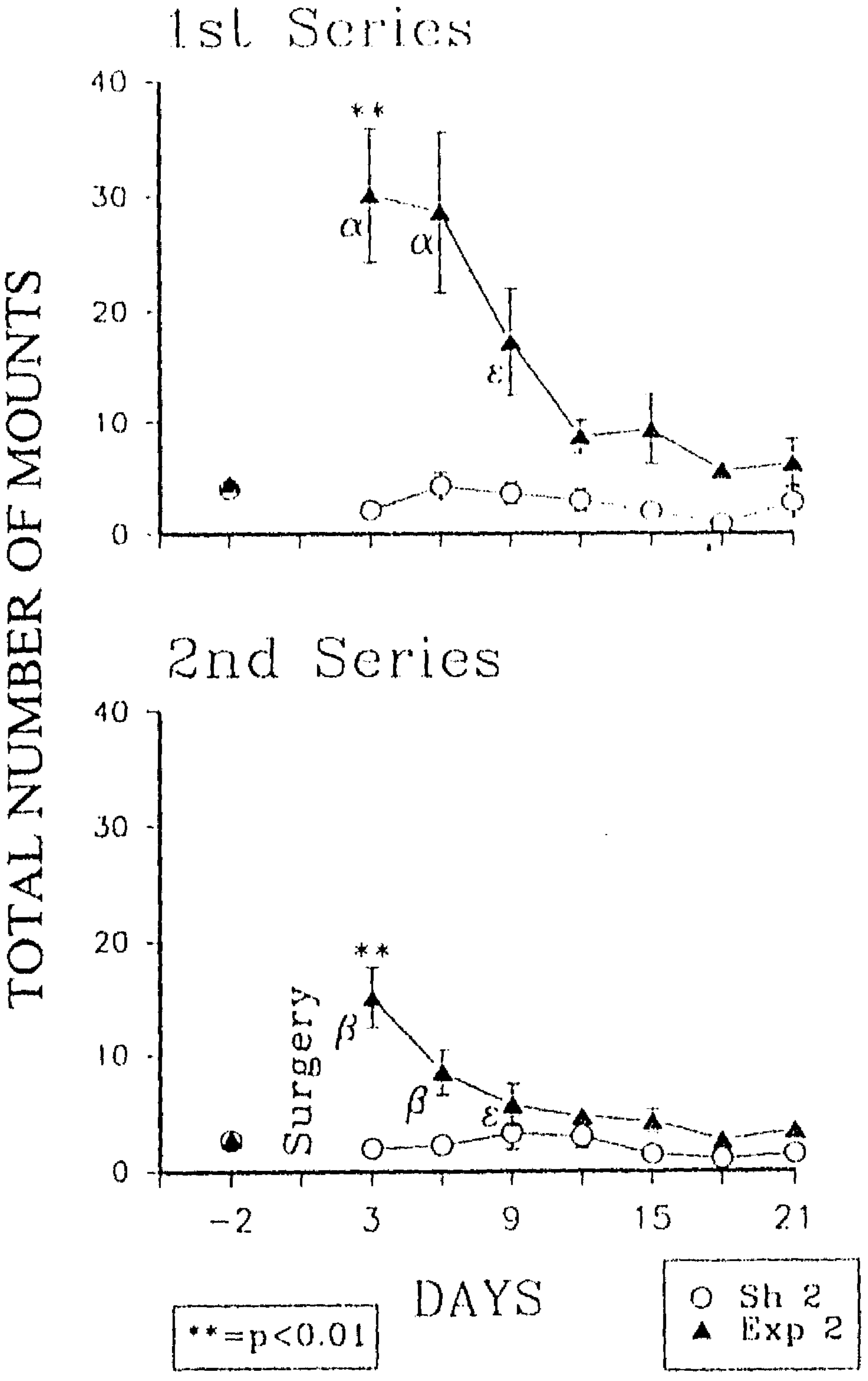

FIG. 1. Total number of mounts during pre- and postsurgery tests of two consecutive ejaculatory series. As compared with sham (Sh 2) animals, in Exp 2 this parameter was significantly increased on day 3 after bilateral sectioning of the pelvic nerve viscerocutaneous branch. Sh 2 (n = 6); Exp 2 (α = 11, β = 13, ϵ = 16, otherwise n = 17). In all figures but 6 and 7 the meaning of the asterisks are in relation with post hoc Tukey test analyses.

The intromission latency (Fig. 2) appeared to be higher for Exp 2 males in both ejaculatory series during the first three tests after surgery. However, ANOVA's did not reveal reliable changes in this measure.

The ANOVAs for the total number of intromissions (Fig. 3) revealed a significant effect of surgery [series I: $F(1, 21) = 10.12$, $p < 0.005$; series II: $F(1, 21) = 12.60$, $p < 0.005$]. There was no main effect of tests for this measure, but the surgery–test interaction was reliable [series I: $F(6, 126) = 3.85$; series II: $F(6, 126) = 3.02$, $p < 0.01$]. Tukey tests revealed that the number of intromissions for Exp 2 was significantly decreased on days 3 and 6 in series I and on day 3 and 9 in series II.

As shown in Fig. 4, the number of intromissions per minute was also temporarily reduced by PvN VC transection [series I: $F(1, 21) = 12.42$, $p < 0.001$; series II: $F(1, 21) = 6.16$, $p < 0.05$]. There was a reliable change across tests for this parameter [series I: $F(6, 126) = 4.63$, $p < 0.001$; series II: $F(6, 126) = 4.99$, $p < 0.001$], but the surgery–test interaction were significant only for the second series, $F(6, 126) = 2.24$, $p < 0.05$. Post hoc comparisons showed a significant decrement in both series only for day 3 postsurgery.

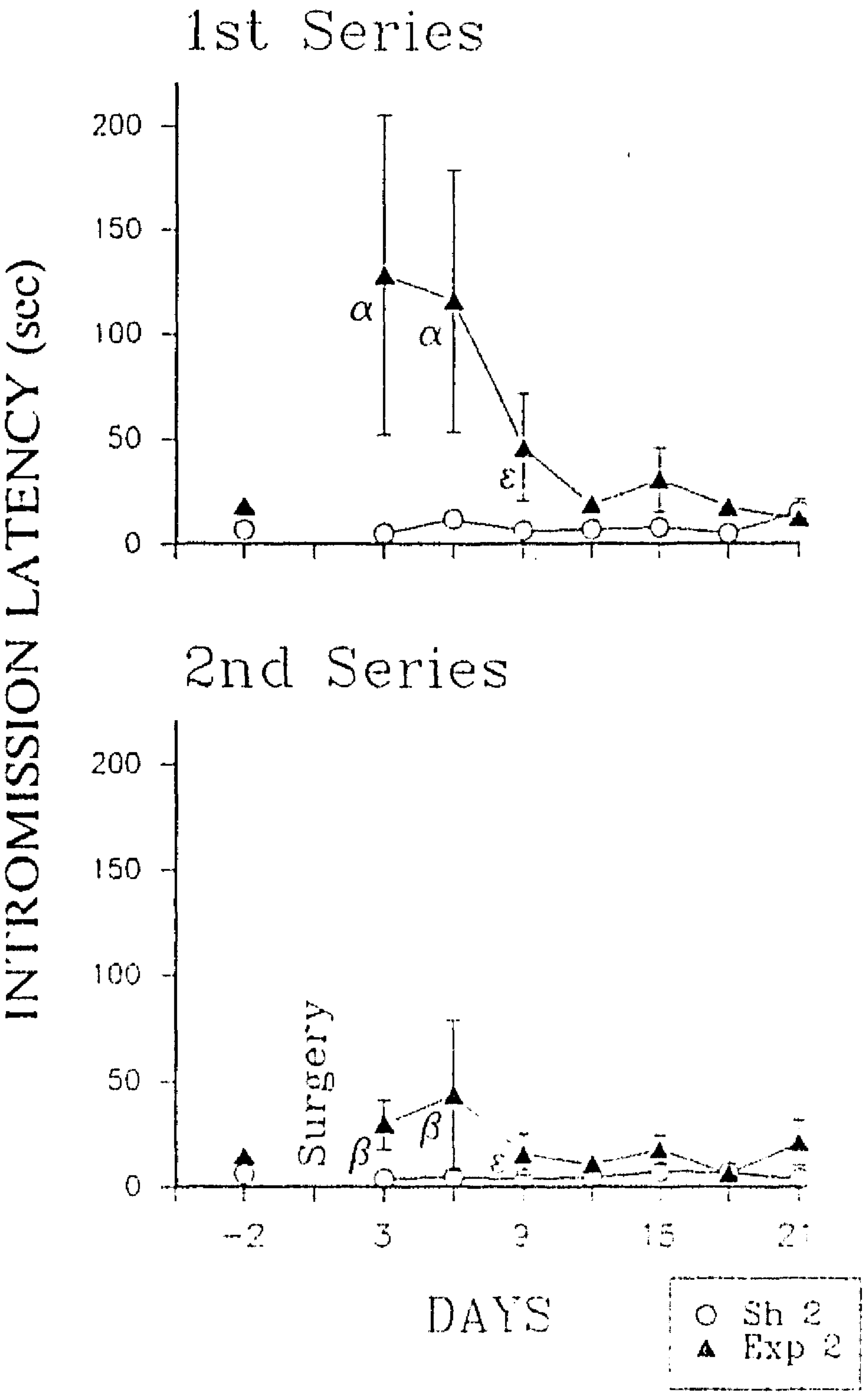

FIG. 2. Intromission latency during pre- and postsurgery tests of two consecutive ejaculatory series. The apparent difference between groups was not reliable for any postsurgery test. Sh 2 ($n = 6$); Exp 2 ($\alpha = 11$, $\beta = 13$, $\epsilon = 16$, otherwise $n = 17$).

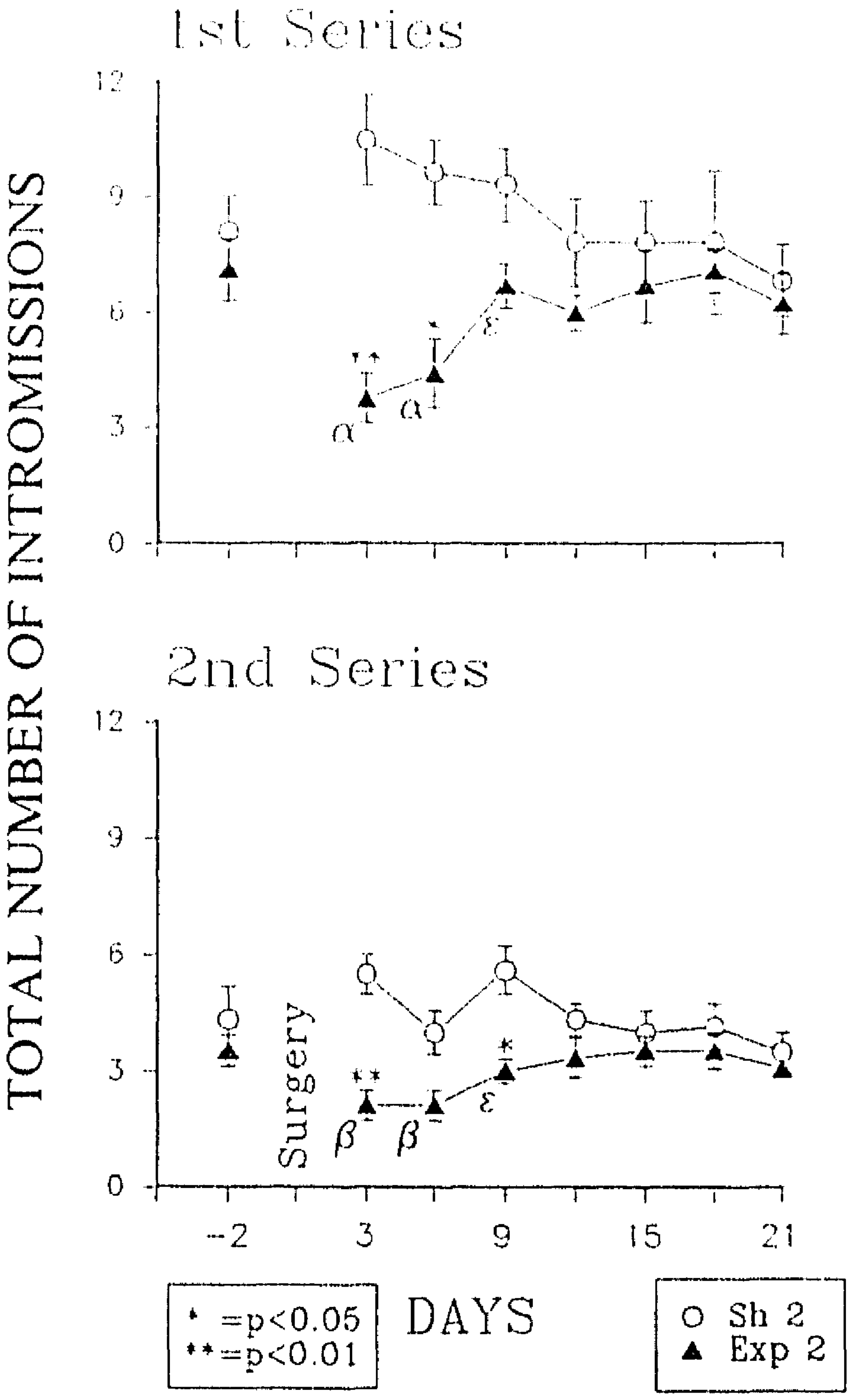

FIG. 3. Total number of intromissions during pre- and postsurgery tests of two consecutive ejaculatory series. After surgery, Exp 2 animals showed a decrement which was significant on days 3, 6, and 9. Sh 2 ($n = 6$); Exp 2 ($\alpha = 11$, $\beta = 13$, $\epsilon = 16$, otherwise $n = 17$).

The intromission ratios, which reflect erectile function, were reliably but transiently reduced (Fig. 5). Thus, the effects of surgery [series I: $F(1, 21) = 28.15$, $p < 0.001$; series II: $F(1, 21) = 20.94$, $p < 0.001$], tests [series I: $F(6, 126) = 8.89$, $p < 0.001$; series II: $F(6, 126) = 8.68$, $p < 0.001$], and the interaction between surgery and tests [series I: $F(6, 126) = 2.50$, $p < 0.05$; series II: $F(6, 126) = 2.74$, $p < 0.05$] were significant. Post hoc Tukey tests of this measure indicated significant decrements for Exp 2 males on days 3 and 6 of series I and on day 3 of series II (Fig. 5).

As shown in Fig. 6, the percentages of males depositing seminal plugs were reduced in most postsurgery tests, although Fisher exact probability test indicated a reliable group difference only on day 18 of series I and on days 9 and 18 of series II. The figure also shows that the percentage of males displaying the ejaculatory pattern was not significantly reduced by PvN VC transection.

Figure 7 presents means ± SEM of seminal plug weight for series I and II. ANOVA revealed that only in the second series,

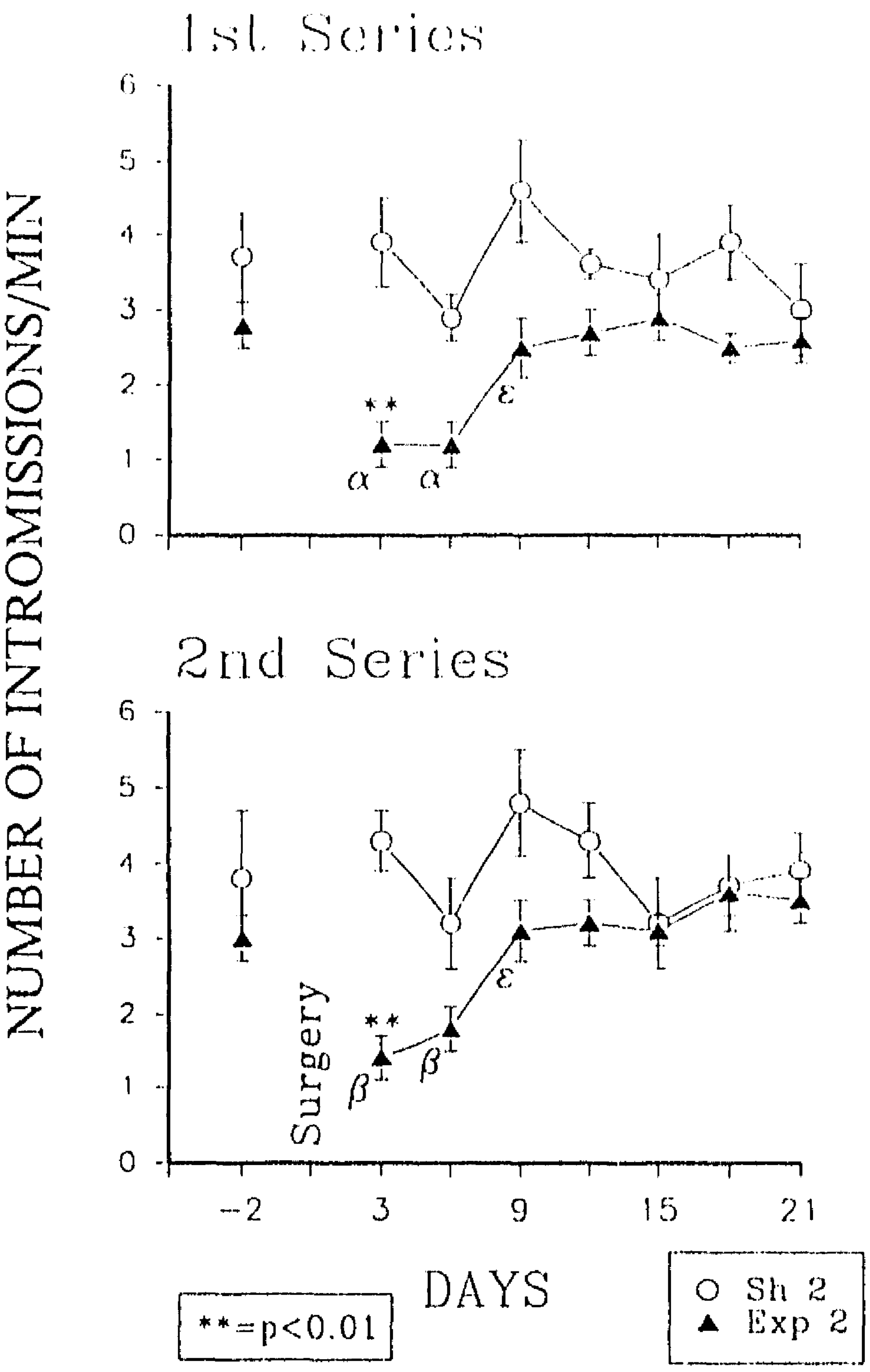

FIG. 4. Number of intromissions per minute performed during pre- and postsurgery tests of two consecutive ejaculatory series. After surgery, Exp 2 male rats showed a decrement which was significant on day 3. Sh 2 ($n = 6$); Exp 2 ($\alpha = 11$, $\beta = 13$, $\epsilon = 16$, otherwise $n = 17$).

surgery caused a significant reduction in plug weight, $F(1, 21) = 8.23$, $p < 0.01$. F-values for tests and interaction of surgery–tests were not reliable for this measure.

DISCUSSION

In contrast with the earlier work of Lodder and Zeilmaker (20), the results of the present study show that pelvic nerve transection transiently alters copulatory behavior in a way that reflects apparent changes in penile erection and ejaculatory function. Thus, PvN VC branch transection produced increments in the intromission latency and the total number of mounts, and decrements in the total number of intromissions, intromission ratio, percentage of ejaculations with plug, and weight of seminal plugs. These deficits were expected on the basis of previous anatomical and physiological studies (9,10), and they cannot be attributed to traumatic effects of surgery: sham males and those with PvN SM branches transected experienced similar surgical trauma without apparent effect. Our discussion, therefore, focuses on compensatory mechanisms underlying the rapid recovery of function after VC branch section.

The intromission ratio is sensitive to treatments that affects a male's erectile potential or penile sensitivity (30). The highly significant reduction in this ratio after section of the VC branches, as well as the increment in the number of mounts and the intromission latency and the reduction on the number of intromissions per minute, all imply interference with mechanisms of erection. The recovery in these parameters may be understood in light of recent observations of Dail et al. (9), who have described rearranged synaptic connections within the autonomic pelvic ganglion after denervation. Dail et al. (10) found that stimulation of the hypogastric nerve had no effect on the cavernous pressure of male rats whose pelvic nerves had been transected within the preceding 24 h. However, beginning 3 days after pelvic nerve section, and for at least 3 months after that, hypogastric nerve stimulation reliably increased cavernous pressure. The authors suggested that the reinnervation in the pelvic plexus may involve the sprouting of hypogastric nerve terminals. We propose that 21 days after surgery in the present study, the

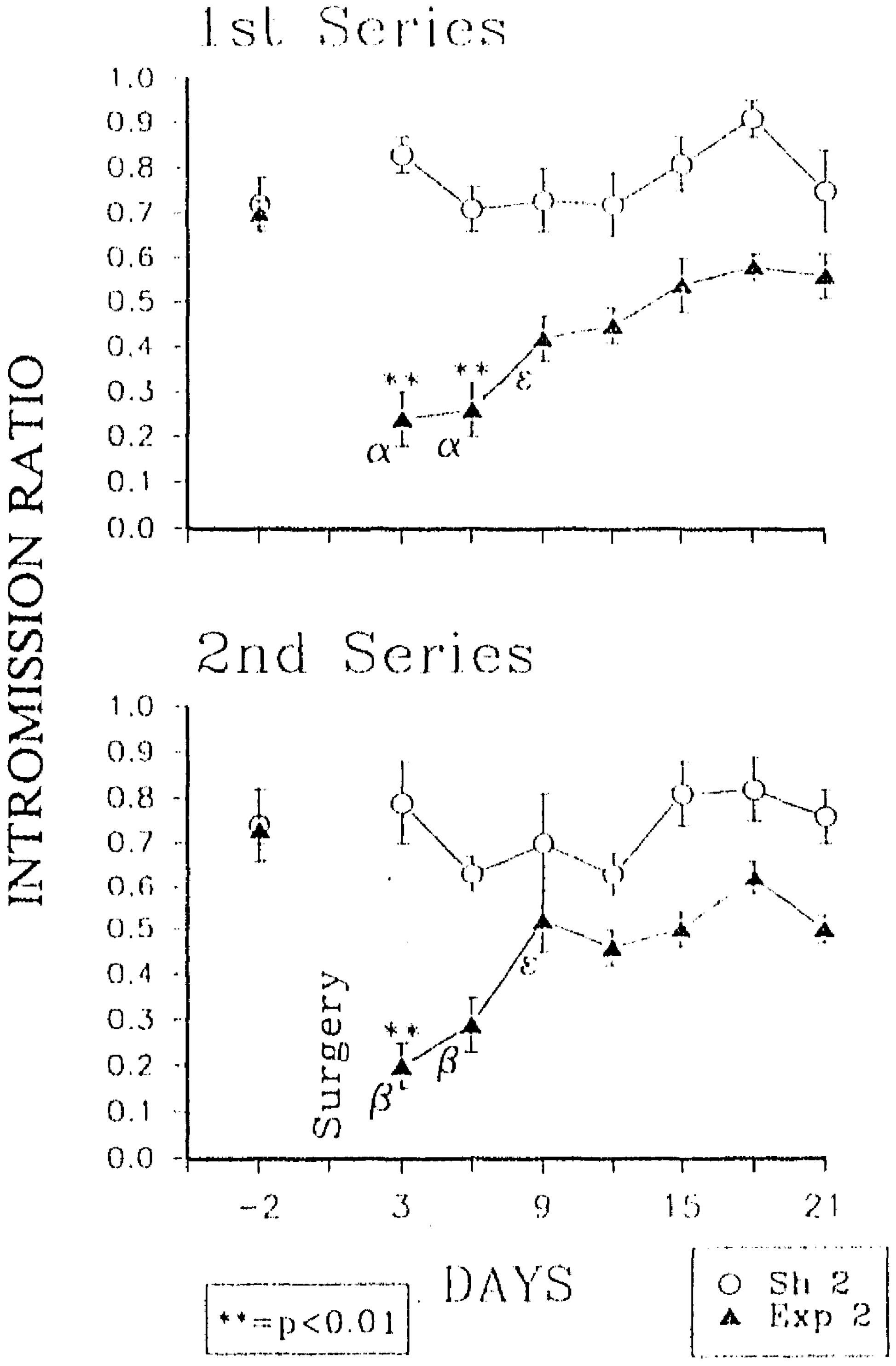

FIG. 5. Intromission ratio calculated from the number of mounts (NM) and intromissions (NI): NI/(NM+NI) obtained during pre- and postsurgery tests of two consecutive ejaculatory series. After surgery it was reliably reduced in Exp 2 animals. Sh 2 ($n = 6$); Exp 2 ($\alpha = 11$, $\beta = 13$, $\epsilon = 16$, otherwise $n = 17$).

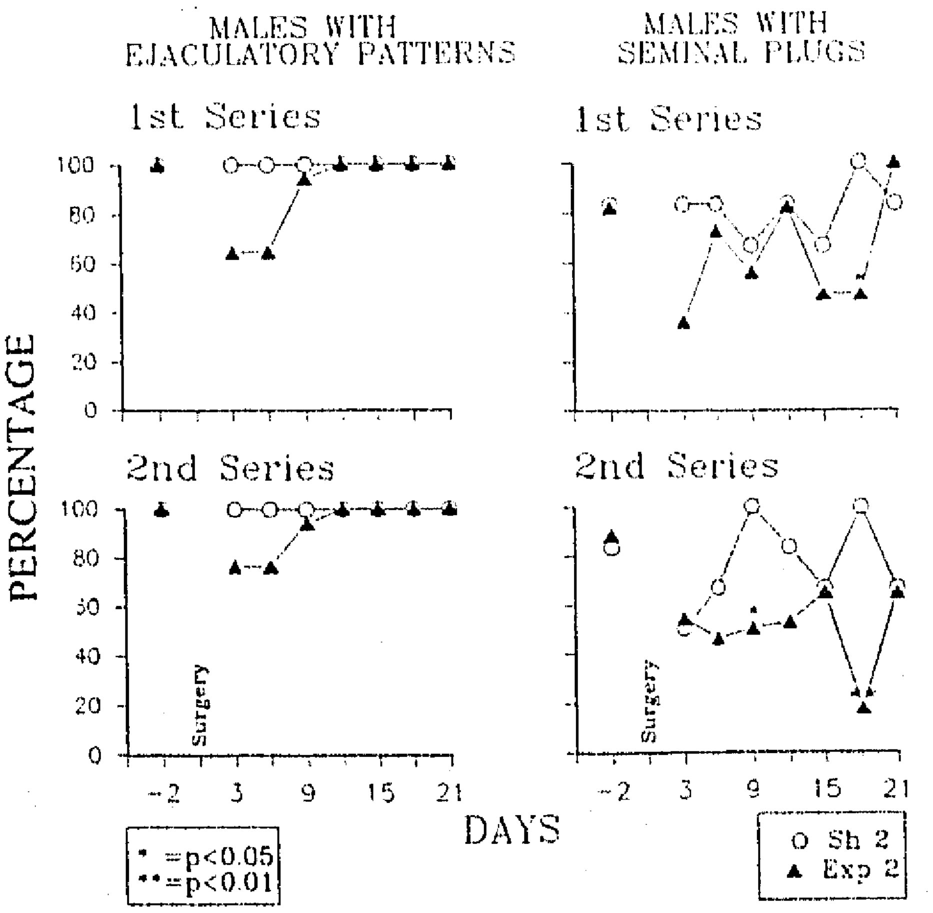

FIG. 6. Percentage of males displaying ejaculatory patterns (left) and depositing seminal plugs (right) during two consecutive ejaculatory series. The reduction in the ejaculatory patterns of Exp 2 animals was not statistically reliable. Percentage of Exp 2 males depositing seminal plugs was reduced, especially in the second series. Sh 2 (n = 6); Exp 2 (n = 17).

VC nerve-transected animals recovered copulatory efficiency through a similar compensatory plastic mechanism.

In other species [cat (1), rhesus monkeys (13), rat (17)], genital deafferentation provokes aberrant mounting behavior, probably by interference with tactile sensation. In our study, although the PvN VC innervates perianal skin and the glabrous skin of the scrotum (22), no aberrant mounts were observed after its transection. The tactile sensations of this area probably are related to defecation functions but not to reproductive activities. However, VC branch-denervated animals presented throughout the study an increment in the total number of mounts (Fig. 1) and number of mounts per minute (data not presented). This increment in mounting should not be viewed as an increase in sexual motivation, but rather to the failure of erection, and, hence, to the absence of the brief refractoriness induced by each intromission (4,5,11,12,16,31). Thus, when a normal male rat is prevented from intromitting by occluding the vagina of the stimulus female, then mounts are more frequent (11). Furthermore, incomplete penile erection due to pudendal nerve sectioning provokes a similar mounting increment (29).

PvN VC transection also produced a reduction in the weight of seminal plugs, which was more noticeable in the second series, and in percentage of males delivering a plug (see Figs. 6 and 7). These effects may reflect that autonomic fibers projecting to structures that participate in seminal emission travel not only via the hypogastric nerve (18) but also via the VC branch of the PvN. In this regard it is important to emphasize that the pelvic nerve carries a significant number of pre- and postganglionic sympathetic as well as parasympathetic fibers (14). According to Bacq (3), after sympathetic denervation of the genital organs, rats and guinea pigs do not produce seminal discharge. Root and Bard (28) described that cats did not present seminal discharge after abdominal sympathetic chain lesions that included the inferior mesenteric ganglia. In dogs, prostatic contractions in the resting state at rest can be obtained with electrical stimulation of the pelvic and/or hypogastric nerve (33). So, as is already well established, sympathetic fibers play an important role in the mechanisms for seminal discharge. The PvN carries a significant number of sympathetic as well as parasympathetic fibers (14) that supply most of the neurons of the main penile nerve, the major autonomic source to the corpus spongiosum and bulbourethral glands (8). These fibers that travel in the PvN VC branch probably play an important role in seminal emission. However, further analyses are necessary to support this interpretation.

ACKNOWLEDGEMENTS

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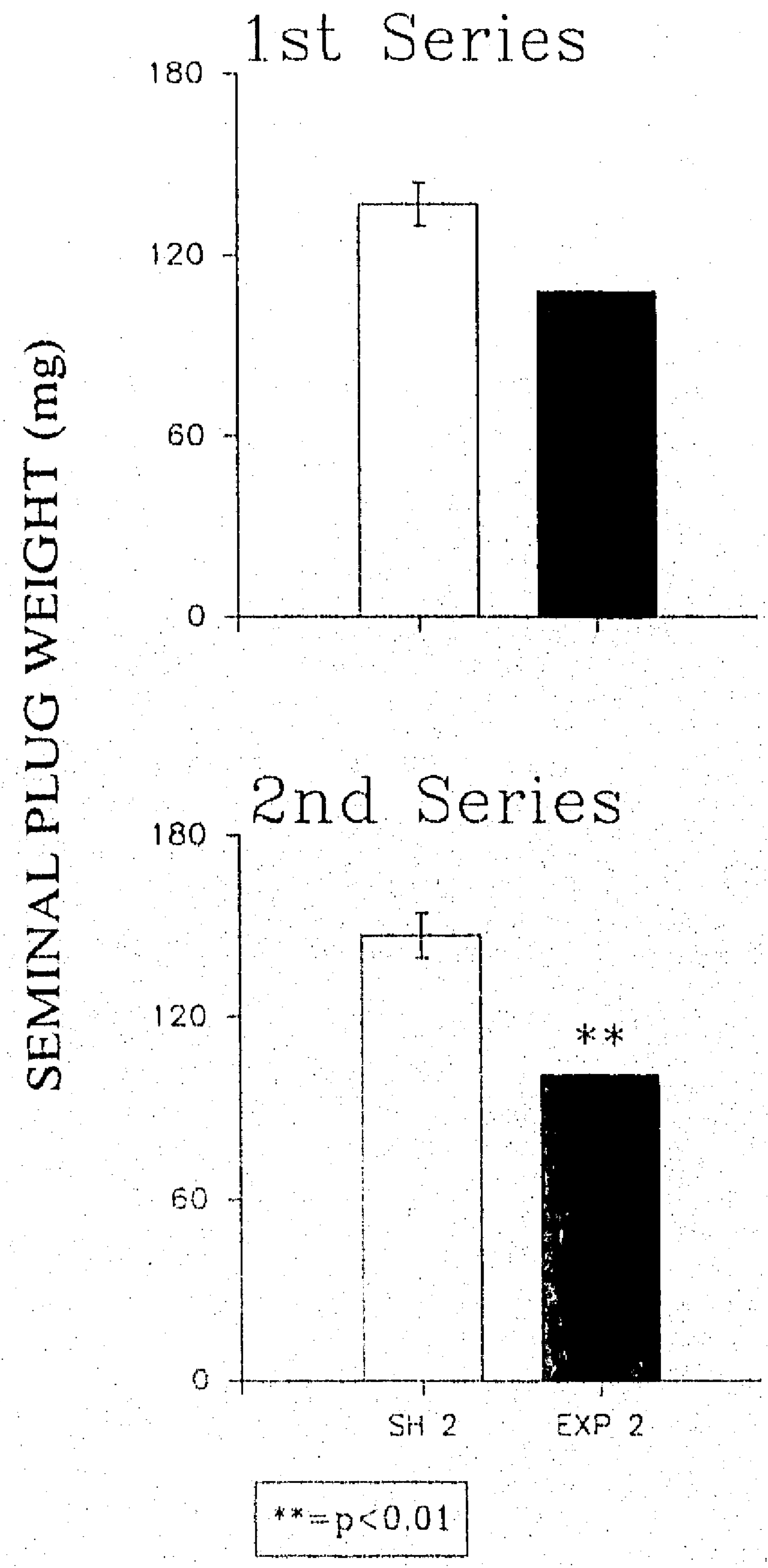

FIG. 7. Seminal plug weight of sham (Sh 2) and experimental (Exp 2) animals. Figure shows means ± SEM from two consecutive ejaculatory series. Bilateral transection of the viscerocutaneous branch of the pelvic nerve (Exp 2) produced a significant reduction only in the second ejaculatory series. Sh 2 (n = 6); Exp 2 (n = 17).

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Striated Muscles, Scent Glands and Sacral Nerves Associated with the Vaginal Tract of the Rabbit

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Running title: VAGINAL MUSCULATURE IN THE RABBIT

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The European rabbit *(Oryctolagus cuniculus)* continues to play an important role in the study of mammalian reproductive function. This is due in part to its suitability as a laboratory animal, and in part to its renowned reproductive capacity. This capacity is based on a number of specializations which not only provide a particularly good opportunity for the study of reproductive processes, but also make an interesting contrast to the rat and cat - two other well-studied laboratory species- from a functional and anatomical point of view (Greene, 1968; Hebel and Stromberg, 1986; Crouch and Lackey, 1969; Martin et al., 1974).

Several behavioral and physiological specializations are of particular relevance to the present study. The rabbit is an induced ovulator (Ramírez and Lin Soufi, 1994), and one intromission by the male accompanied by a brief series of rapid pelvic thrusts is sufficient to ensure ejaculation, ovulation and pregnancy (Soto et al., 1984; Ramírez and Lin Soufi, 1994; own observations). The doe normally gives birth in a separate nursery burrow, and presumably to avoid predation (Hudson and Distel, 1982; 1989), but also because the umbilical cord usually ruptures in the long vagina, the pups are expelled in rapid succession. In domestic breeds, the birth of as many as 14 pups is achieved within 10 to 15min (Fuchs and Darwood, 1980; Hudson et al., 1995).

In addition, chemical communication plays and important part in the rabbit's reproductive behavior (Mykytowycz, 1972; Bell, 1980; Hudson and Distel, in press). In both sexes, urine, feces, and secretions from at least three sets of glands in the pelvic area - the anal, the inguinal, and in the male, the preputial glands (Coujard, 1947; Montagna, 1950; Kühnel, 1971; Holtz and Foote, 1978)- all appear to have specific communicatory functions.

Given the rabbit's well-established position in the reproductive literature, it is surprising that no comprehensive description of pelvic anatomy is available for this species. Although the early studies of Langley and Anderson (1896a,b) provide valuable information on the innervation, and the books by Barone et al. (1973) and Popesko (1979) include good general descriptions, none of these authors describe the innervation and musculature of the vaginal tract in the detail necessary for understanding its various functions.

It is therefore the purpose of this study to extend these reports by giving a more detailed description of structures associated with the vaginal tract and to consider their posible contribution to the rabbit's remarkable reproductive efficiency.

MATERIALS AND METHODS

Animals

A total of eight sexually mature, virgin, chinchilla-breed female rabbits weighing between 3.0 and 3.5 kg was used. They were individually housed in the Tlaxcala colony and maintained under LD 16:8 (lights on at 0600) at 20 ± 2° C, with food (Purina® rabbit chow) and water available continuously. Four animals were used for dissection, and four for electrical stimulation of the muscles after which they also were dissected.

Dissection

All animals were deeply anesthetized with 0.7 g/kg urethane administered as a 20% solution i.p., and at the end of the examination animals were euthanized with an overdose into the ear marginal vein. For dissection, animals were fixed in a dorsal supine position and a midline incision was made from the clitoral sheath to the sternum. Using a dissecting microscope, the main structures were identified and drawings were made concentrating on structures associated with the vaginal tract. As a considerable part of the tract was covered by the pubic bone it was necessary to remove this during the dissection (cf. Figs. 1 and 2). To prevent bleeding, blood vessels were ligated where necessary.

The dissection included following the main pathways of the sacral nerves from their point of exit at the vertebral column to target structures. For this it was necessary to open the dorsal surface of the animal, at which point it was sacrificed before proceeding with dissection of the musculature surrounding the vertebrae.

Electrical Stimulation

Uni- and bilateral electrical stimulation of muscles was performed to better define individual structures. Bipolar electrodes placed 5 mm apart were used. They were made from stainless steel wires electrically insulated except for 1 to 2 mm scratched free at the tip. Stimulation was provided by square pulses of 0.1 msec duration delivered through a Grass SIU5 stimulus isolation unit connected to a Grass S44 stimulator. Intensities used ranged from 0.1 to 10 v, and frequencies from 1 to 10/sec. Stimuli were administered to each muscle several times while watching for movement in or modification of shapes' structures.

Nomenclature

Where possible, structures were named according to Nomina Anatomica Veterinaria (1983), and Table 1 compares the nomenclature for muscles used in this paper with terms used in previous studies of the rabbit.

--- Insert Table 1 about here ---

OBSERVATIONS

Main Structural Features

Viewed ventrally, the vaginal tract could be divided broadly into two regions - a rostral section approximately 6 cm long located beneath the pubic bone and extending approximately 3 cm beyond its rostral edge, and a caudal section protruding approximately 3 cm beyond the bone's caudal edge (Figs. 1 and 3). The urinary bladder was located immediately rostral to the pubic bone, and beneath it the uterine horns converged to form the double cervix (cervix duplex; Barone et al., 1973; Schley, 1985) characteristic of the rabbit. The urethra, lying ventral to the vagina, opened into it about midway beneath the pubic bone.

--- Insert Fig. 1 about here ---

Removing the pubic bone exposed first the obturatorius internus muscles, and beneath these the constrictor vestibuli muscles which covered the portion of the vagina located beneath the pubic bone (Figs. 2, 4 and 5A). Beneath the constrictor vestibuli muscles the vaginal venous plexus (plexus venous vaginae; Popesko, 1979) and the vestibular glands (glandulae vestibulares; Barone et al., 1973) formed a complex attached to the urethra for its entire length (Figs. 1 and 4). More deeply still, the coccygeus muscles travelled medially from the innominate bone to the vertebral column. In close relation with the fibers of the coccygeous muscles but travelling from the innominate bone caudally to the vertebral column, the fibers of the pubo- and iliocaudalis muscles could be seen (Figs. 2 and 5A). Running caudally from the ischiatic arc, the bulbospongiosus muscles flanked by the more dorsally-situated ischiocavernosus muscles converged onto a tendon attached to the ventral midline of the vagina and to the clitoris (Fig. 3). Beneath these muscles the constrictor vulvae muscles formed a tough covering over most of the protruded portion of the vagina (Figs. 4 and 5B).

--- Insert Figs. 2 to 5 about here ---

Embedded among the fibers of the constrictor vulvae muscles, the extensive anal glands could be seen, and lying superficially to these and to the constrictor vulvae, the inguinal glands (Figs. 4 and 5B). More caudally still, in the cleft between the vaginal opening and anus, the pouches associated with ducts from the inguinal glands could be found, and in the skin defining the vaginal opening the preputial gland complex was identified (Figs. 1 and 6).

--- Insert Fig. 6 about here ---

Muscles and Nerves

Proceeding in a rostro-caudal direction, the muscles together with their sacral innervation will now be described in more detail.

M. coccygeus. The fibers of this muscle formed a triangular sheet which originated from the medial surface of the innominate bone and spread out medially in a ventro-dorsal direction to insert without tendinous appearance onto the transverse processes of the second, third and fourth sacral vertebrae (Figs. 2 and 5A). This muscle appeared to be innervated by a branch of the S2 spinal nerve after anastomotic connection with S3 (Fig. 7). Unilateral electrical stimulation of the muscle produced tension in it and ipsilateral abduction of the tail whereas bilateral stimulation produced rigidity in the tail and dorsal abduction moved it in a dorso-ventral direction. Movement of the vagina or other associated structures was not observed.

--- Insert Fig. 7 about here ---

M. pubocaudalis. The fibers of this muscle originated from the caudal portion of the medial face of the innomiate bone. They travelled rostro-caudally and ventro-dorsally, passing close to the lateral walls of the vagina and rectum, to insert by a strong tendon onto the transverse processes of the fourth coccygeal vertebra (Figs. 2 and 5). This muscle appeared to be innervated by a branch of the S3 spinal nerve after anastomotic connection with S2 (Fig. 7). Unilateral electrical stimulation of the muscle resulted in slight flexion of the base of the tail and in clearly visible movements of the lateral walls of the protruded portion of the vagina and rectum. Bilateral stimulation produced rigidity in the tail but no visible movements in other structures.

M. iliocaudalis. The fibers of this muscle arose from the caudal portion of the medial face of the innominate bone at a site immediately adjacent to the pubocaudalis muscle. They ran in close parallel but dorsal to those of the pubocaudalis to insert onto a tendon attached to the transverse processes of the fifth coccygeal vertebra. This muscle also appeared to be innervated by a branch of the S3 spinal nerve after anastomotic connection with S2 (Fig. 7). Unilateral electrical stimulation of the muscle produced strong ipsilateral abduction of the tail and similar movements in the vagina and rectum as for the pubocaudalis muscle. Bilateral stimulation produced rigidity and shivering in the tail but no visible movements in other structures.

M. obturatorius internus. This muscle originated from the internal face of the pubic bone along the pubic symphysis. The triangular sheet of fibers travelled laterally in a ventro-dorsal direction to converge onto a strong tendon which inserted onto the major trochanter of the femur (Figs. 2 and 5A). Although the innervation of this muscle was not investigated in the present study, the obturatorius internus and externus are reported to be innervated by branches of the L5, L6, and L7 spinal nerves (Langley and Anderson, 1896b). Unilateral electrical stimulation of the muscle provoked movement in the ipsilateral leg.

M. bulbospongiosus and ischiocavernosus. These two muscles could be clearly identified as separate sheets of fibers - the medial sheets forming the bulbospongiosus and the lateral sheets the more dorsally located ischiocavernosus muscles (Figs. 3 and 5B). Although they appeared at first sight to form just one structure, careful dissection and unilateral electrical stimulation revealed two distinct pairs of muscles. Both pairs arose from the caudal border of the ischiatic arc and ran rostro-caudally and ventro-dorsally to insert onto a common tendon connected to the ventral midline of the vagina and to the cartilaginous ligamentum suspensorium clitoridis (Barone et al., 1973). The two muscle pairs appeared to be innervated by separate branches of the S2 spinal nerve after anastomotic connection with S1 and S3 (Fig. 7). Unilateral electrical stimulation of each muscle - particularly of the bulbospongiosus - produced ipsilateral movements of the clitoral sheath whereas bilateral stimulation resulted in retraction and rigidity of the sheath and retraction of the labia to expose the vaginal entrance.

M. constrictor vestibuli. This muscle was composed of fibers which originated along the body of the fourth sacral and first coccygeal vertebrae (Figs. 4 and 5A) and ran dorso-ventrally to cover the venous plexus, the vestibular glands and the vagina to the caudal border of the pubic bone. Rostrally it, together with its contralateral counterpart, formed a fine, interlacing sheet of fibers over the venous plexus, and caudally a strong sling-like structure attached to the ventral midline of the vagina beneath the most rostral portion of the bulbospongiosus and ischiocarvenosus muscles. It appeared to be innervated by a branch of the S3 spinal nerve after anastomotic connection with S2 (Fig. 7). The branch travelled along the internal surface of the muscle, running in a rostro-caudal direction and branching along its path. Bilateral electrical stimulation of these muscles resulted in compression of the vagina and rectum, and at the level of the urethra, compressed this, the venous plexus, and the vestibular glands against the vaginal wall. Unilateral stimulation provoked ipsilateral movements in these structures.

M. constrictor vulvae. This muscle formed a thin but tough tunnel-like sheet enclosing most of the protruded portion of the vagina and rectum (Figs. 4 and 5B). It was attached to the body of the second and third coccygeal vertebrae and joined at the midline of the ventral vaginal wall in a similar way to the caudal portion of the constrictor vestibuli muscle. Embedded among its fibers were the anal glands (Figs. 4 and 5B). It appeared to be innervated by a separate branch of S3 to the constrictor vestibuli (Fig. 7) which also travelled along the internal surface of the muscle in a rostro-caudal direction, branching along its path. Bilateral electrical stimulation of these muscles provoked retraction of the clitoral sheath, contraction of the vaginal and rectal walls, and compression of the anal glands whereas unilateral stimulation provoked ipsilateral movements in these structures.

Scent Glands

Although the three sets of glands described here may have others, as yet unspecified functions, given their likely role in chemical communication they will be referred to as scent glands.

Anal glands (Glandulae analis). As mentioned above, these were found embedded in the constrictor vulvae muscles, and starting about 2 cm from the anus, ran caudo-rostrally in the sulcus formed by the vagina and rectum (Figs. 1, 4 and 5B). Each gland was approximately 2.5 cm long and 0.6 cm wide, and consisted of either two or three clusters of brownish lobes divided by connective tissue related with the constrictor vulvae, or of just one elongated, apparently continuous series of lobes. From the gross dissection they appeared to be innervated by a branch of the S2 spinal nerve after anastomosis with S1 and S3 (Fig. 7) - that is, by a different nerve to the constrictor vulvae muscle - which ran rostro-caudally, branching along the glands' length.

Inguinal glands (Glandulae inguinales). These were located superficially over the anal glands and constrictor vulvae muscles about 2 cm from the vaginal opening, and had ducts leading to deep pouches of hairless skin situated about 1 cm more caudally on either side of the anus and vagina (Figs. 1, 4, 5B and 6). The glands consisted of a superficial, pale-colored spherical lobe about 0.4 cm in diameter, and an adjacent, deeper-lying, dark brown lobe approximately 1.5 cm long and 0.4 cm wide. Like the anal glands, the inguinal gland complex appeared to be innervated by a separate branch of the S2 spinal nerve after anostomotic connections with S1 and S3 (Fig. 7).

Preputial glands (Glandulae preputiales). These were inconspicuous and could have been easily overlooked. Embedded in the vulva labia, they formed a necklace-like double ring around the entrance of the vagina although they were most concentrated dorsally (Fig. 6). Each gland was associated with one or more long, stiff hairs and contained a thick, whitish secretion. Similarly to the anal and inguinal glands, innervation appeared to be by a branch of the S2 spinal nerve after anastomosis with S1 and S3 (Fig. 7).

DISCUSSION

This study provides a more complete description of the gross anatomy and sacral innervation of the striated muscles and scent glands associated with the vaginal tract of the rabbit than was previously available. While the supine, ventral view favored here produces some distortion in the exact size and location of organs, we consider that it provides a perspective helpful in thinking about these structures in functional terms, and thus represents a useful addition to the more naturalistic lateral views given by Barone et al. (1973) and Popesko (1979).

Although our observations of muscles are largely consistent with previous descriptions, none of the earlier accounts gives the exact origin and insertion of the muscles associated with the vaginal tract, nor provides a description of their possible action in modifying other pelvic structures. Furthermore, the constrictor vestibuli and vulvae - the two muscles most closely associated with the vagina - have been previously only briefly mentioned, if at all.

With regard to the scent glands, our findings accord well with earlier previous descriptions of the anal (Coujard, 1947; Mykytowycz, 1966a; Kühnel, 1971) and inguinal glands (Coujard, 1947; Montagna, 1950; Mykytowycz, 1966b). However, although the preputial glands have been previously identified in the male (Barone et al., 1973; Popesko, 1979), and their morphology described in some detail by Holtz and Foote (1978), to our knowledge this is the first time they have been identified in the female. This is perhaps surprising given the well-known presence of similar glands in female rats and mice and the many behavioral, endocrinological, and biochemical studies interest in them has generated (Bronson and Caroom, 1971; Gawienowski et al., 1975; Lucas et al., 1982).

Although the pattern of sacral innervation found here is broadly consistent with the descriptions of Langley and Anderson (1896a,b), this needs to be more closely specified using finer electrophysiological and histological methods. Nevertheless, a first comparison with the rat and cat may be made. While taking into account the complexities of pelvic innervation in the rabbit due to the many small ganglia (Langley and Anderson, 1896b) and anastomotic connections, the following parallels can be drawn. As already suggested by the nomenclature of Barone et al. (1973), the branch of S2 innervating the urethra, external anal sphincter, bulbospongiosus and ischiocarvenosus muscles, clitoral sheath, and the three sets of scent glands (Fig. 7) may be regarded as broadly equivalent to the pudendal nerve in the rat and cat (Bradley and Teague, 1972; McKenna and Nadelhaft, 1986; Pacheco et al., 1989). Furthermore, the bifurcated branch of S3 innervating visceral structures such as the urinary bladder, cervices, vagina and rectum on the one hand, and the pubo- and iliocaudalis muscles, the constrictor vestibuli and vulvae muscles, and the perineal skin on the other, appears to correspond to the viscero-cutaneous and somato-motor branches of the pelvic nerve described in the rat (McKenna and Nadelhaft, 1986; Pacheco et al., 1989; Martínez-Gómez et al., 1992).

However, in other respects the vaginal tract of the rabbit is very different - above all in its relative length. Whereas in the rat (Greene, 1968; Olds and Olds, 1979) and cat (Crouch and Lackey, 1969) the vagina ends at the caudal edge of the pubic bone, in the rabbit it protrudes several centimeters beyond this. Furthermore, whereas in the rat the urethra terminates in an independent external meatus (Olds and Olds, 1979; Hebel and Stromberg, 1986), and in the cat it joins the vagina close to the vaginal orifice (Crouch and Lackey, 1969), in the rabbit it enters the vagina approximately 4cm from the orifice about midway along the vagina's length (Barone et al., 1973).

The vaginal musculature is also rather different. The constrictor vestibuli and constrictor vulvae, so evident in the rabbit, are not present in the rat (Reiner et al., 1980; McKenna and Nadelhaft, 1986), and appear less developed in the cat. In this later species the constrictor vestibuli are reported to arise from fascia of the pars cranialis, and the constrictor vulvae from fascia of the pars caudalis of the external anal sphincter (Martin et al., 1974) rather than from strongly anchoring vertebrae as in the rabbit. With regard to the bulbospongiosus and ischiocarvenosus muscles, whereas these are so slightly developed in the female rat as to be often overlooked (Breedlove and Arnold, 1981; McKenna and Nadelhaft, 1986), in the female cat the ischiocavernosus muscles are half the size of those in the male and the bulbospongiosus muscles do not appear to be present at all (Martin et al., 1974).

Furthermore, only the rabbit appears to posses three such well-developed sets of scent glands in the pelvic area. Whereas neither the rat nor cat are reported to have inguinal glands, the female cat has anal but apparently not preputial glands (Martin et al., 1974; Crouch and Lackey, 1969), and the female rat has preputial but apparently not anal glands (Olds and Olds, 1979; Lucas et al., 1982; Hebel and Stromberg, 1986).

Returning to the reproductive specializations of the rabbit mentioned in the introduction, we may now consider how the anatomical features observed here might relate to these.

With regard to copulation, the well-developed constrictor vestibuli and constrictor vulvae muscles, combined with the action of the pubo- and iliocaudalis muscles, might function to ensure that both male and female receive the stimulation necessary for rapid ejaculation and triggering of hormonal events associated with reflex ovulation and implantation (cf. Pacheco et al., 1989; Martínez-Gómez et al., 1992; 1995). The fact that the male often emits a sharp cry as the penis is withdrawn following ejaculation (own observations) may be associated with strong vaginal pressure.

Furthermore, perigenital stimulation during copulation may be particularly important in the rabbit (cf. Martínez-Gómez et al., 1995). Given the length of the vagina, it is possible that the cervical stimulation important during copulation in the rat (Komisaruk and Wallmann, 1977) is lacking or much reduced. In addition, the well-developed bulbospongiosus and ischiocavernosus muscles may facilitate rapid intromission by positioning and stiffening the vagina and retracting the large clitoral sheath and the labia to expose the vaginal entrance.

With regard to parturition, the complex vaginal musculature may also function to ensure the unusually rapid transit and expulsion of fetuses typical in the rabbit (Fuchs and Darwood, 1980; Hudson et al., 1995).

Although the significance of the female's pelvic anatomy for chemical communication remains unclear, several observations may be of relevance. First, as the urethra enters the vagina so far rostrally, it is possible that the strong musculature of the vaginal tract is used to generate the pressure necessary for the urine spraying demonstrated by estrous females (Bell, 1980; own observations). Furthermore, the preputial glands, aided by the long hairs, may add their contents to the urine as part of female sexual advertisement and marking behavior (cf. Bronson and Caroom, 1971).

The action of the constrictor vulvae muscles in compressing the anal glands may represent the mechanism by which rabbits are able to "voluntarily" regulate the olfactory quality of fecal pellets (Hesterman and Mykytowycz, 1968; Bell, 1980). In what way the inguinal glands are activated, if at all, remains unclear although their superficial location suggests that this may be neural.

Finally, the significance of the venous plexus and vestibular glands should be an interesting subject for future study. Whether, when compressed against the ventral walls of the urethra and vagina by the contraction of the constrictor vestibuli muscles, they release substances providing lubrication during copulation or parturition, alter the pH or other chemical characteristics of the vaginal tract to facilitate sperm migration and survival, or are associated with chemical communication (cf. Masters et al., 1986; Hafez, 1993) remains to be investigated.

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Fig. 6 Lateral view of the preputial glands of a female rabbit which form a necklace-like ring around the entrance to the vagina. Arrows indicate two glands containing whitish secretion and bearing long, stiff hairs; the asterisk marks the inguinal pouch and just above it, an inguinal gland pore.

Fig. 7 Schematic representation of the pattern of innervation of the pelvic area of the female rabbit by spinal nerves L7 to S3. Abbreviations: Abr-Anastomotic branch, Ag-Anal gland, BSP-Bulbospongiosus muscle, CC-Coccygeus muscle, Cs-Clitoral sheath, CVL-Constrictor vulvae muscle, CVS-Constrictor vestibuli muscle, Cx-Cervices, EAS-External anal sphincter, IC-Iliocaudalis muscle, Ig-Inguinal gland, ISC-Ischiocavernosus muscle, PC-Pubocaudalis muscle, Pg-Preputial glands, Ps-Perineal skin, Rc-Rectum, Sci-Sciatic nerve, ST-Semitendinosus muscle, Ub-Urinary bladder, Ut-Urethra, Vg-Vagina.

FIGURE LEGENDS

Fig. 1 Ventral view of the pelvic region of an adult female rabbit drawn approximately to scale showing the relative positions of the main structures associated with the vaginal tract. The pubic bone has been removed, and A and B give the approximate levels of the transverse views shown in Figure 5.

Fig. 2 Ventral view of the pelvic region of an adult female rabbit showing the position of the coccygeus, pubocaudalis, iliocaudalis, and obturatorius internus muscles; S indicates sacral and C coccygeal vertebrae.

Fig. 3 Ventral view of the pelvic region of an adult female rabbit showing the position of the bulbospongiosus and ischiocavernosus muscles.

Fig. 4 Ventral view of the pelvic region of an adult female rabbit after removal of the pubic bone to show the position of the constrictor vestibuli and constrictor vulvae muscles.

Fig. 5 Transverse views of the vaginal tract of an adult female rabbit from rostral (A) to more caudal (B) not drawn to scale. The approximate level of each section is shown in Figure 1.

TABLE 1. Nomenclature of muscles from the Nomina Anatomica Veterinaria used in this paper compared with the nomenclature used in previous studies of the female rabbit.

Nomina Anatomica Veterinaria (1983)	Langley and Anderson (1896)	Barone et al. (1973)	Popesko (1979)
Coccygeus	Coccygeus	Coccygeus	Coccygeus
Pubocaudalis Iliocaudalis	Pubocaudal Iliocaudal	Levator ani	-
Obturatorius internus	Obturator internus	Obturatorius internus	Obturatorius internus
Bulbospongiosus	-	Subischiocavernosus	-
Ischiocavernosus	Ischiocavernosus	Ischiocavernosus	-
Constrictor vestibuli	-	Constrictor vestibuli	-
Constrictor vulvae	External sphincter of the vagina	Constrictor vulvae	Constrictor vulvae

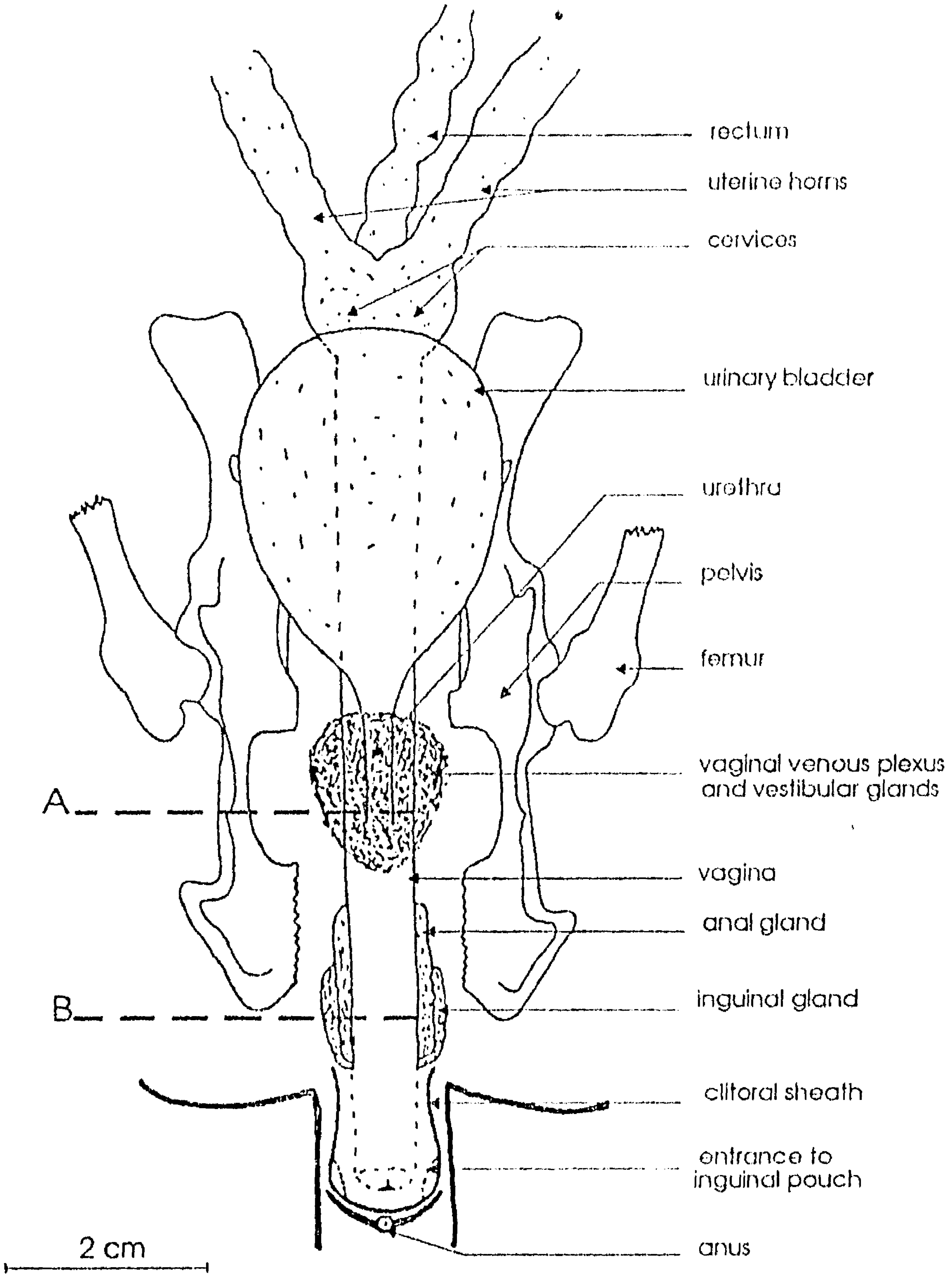


Fig. 1 (Martínez-Gómez et al.)

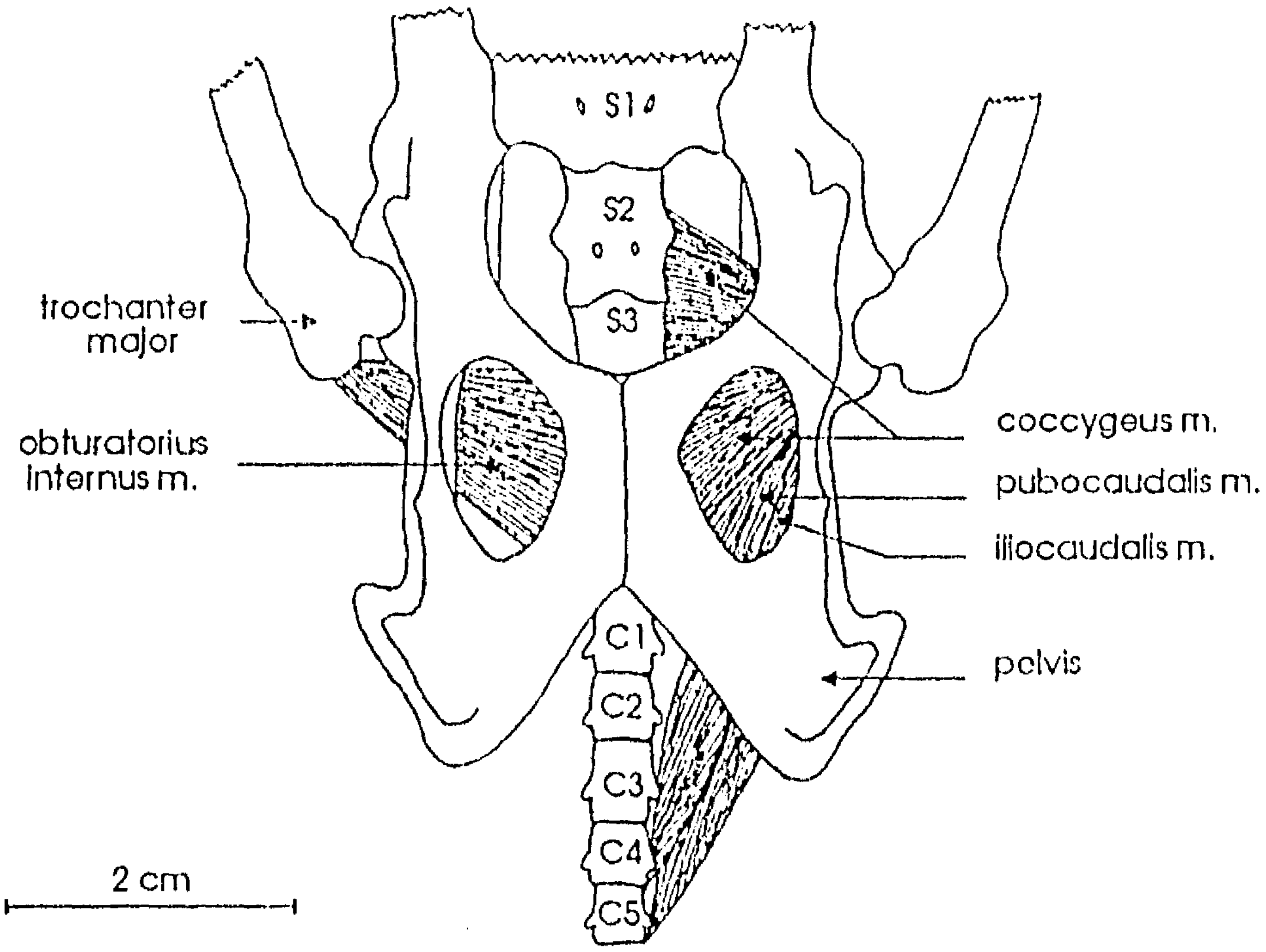
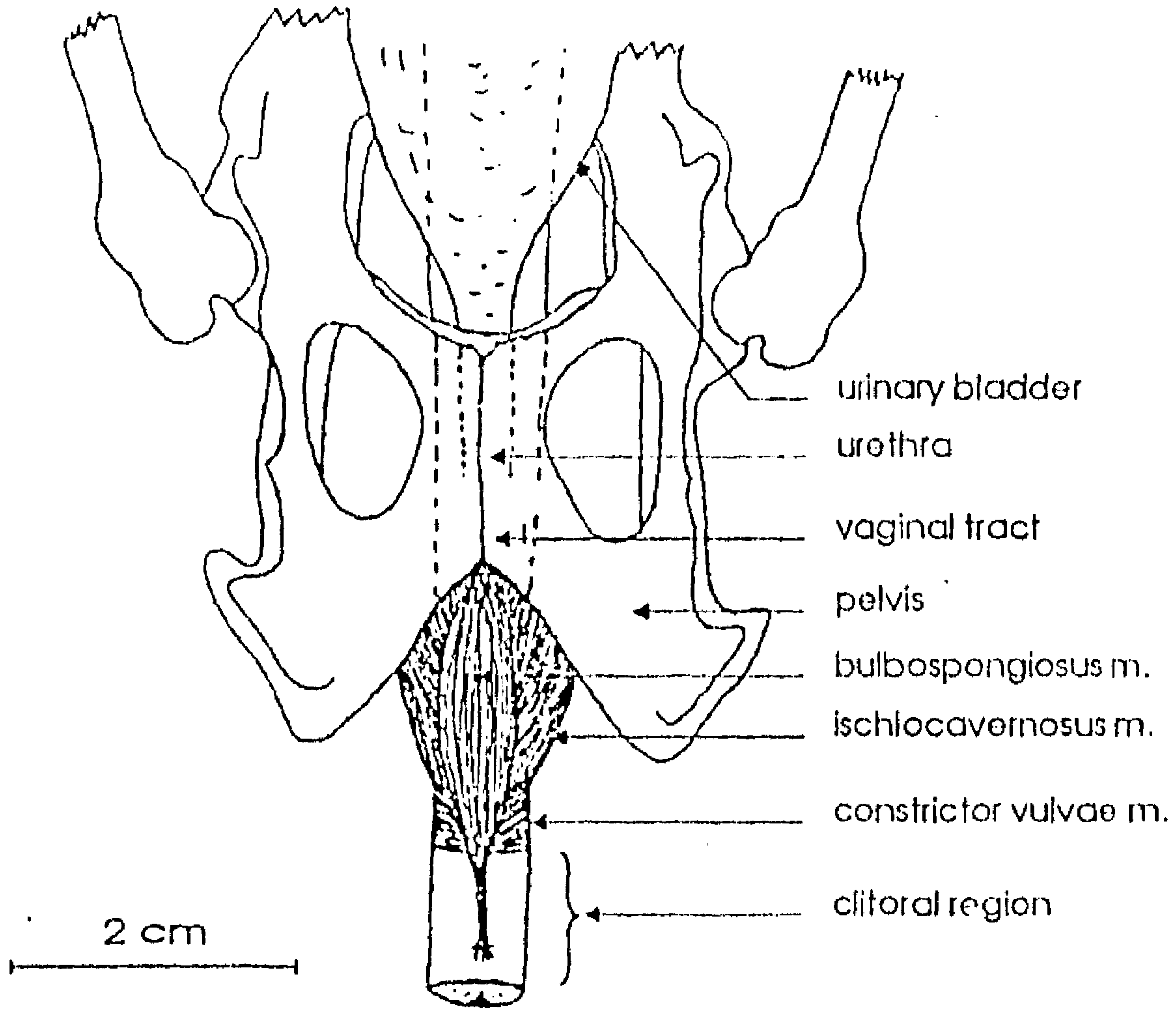


Fig. 2 (Martínez-Gómez et al.)



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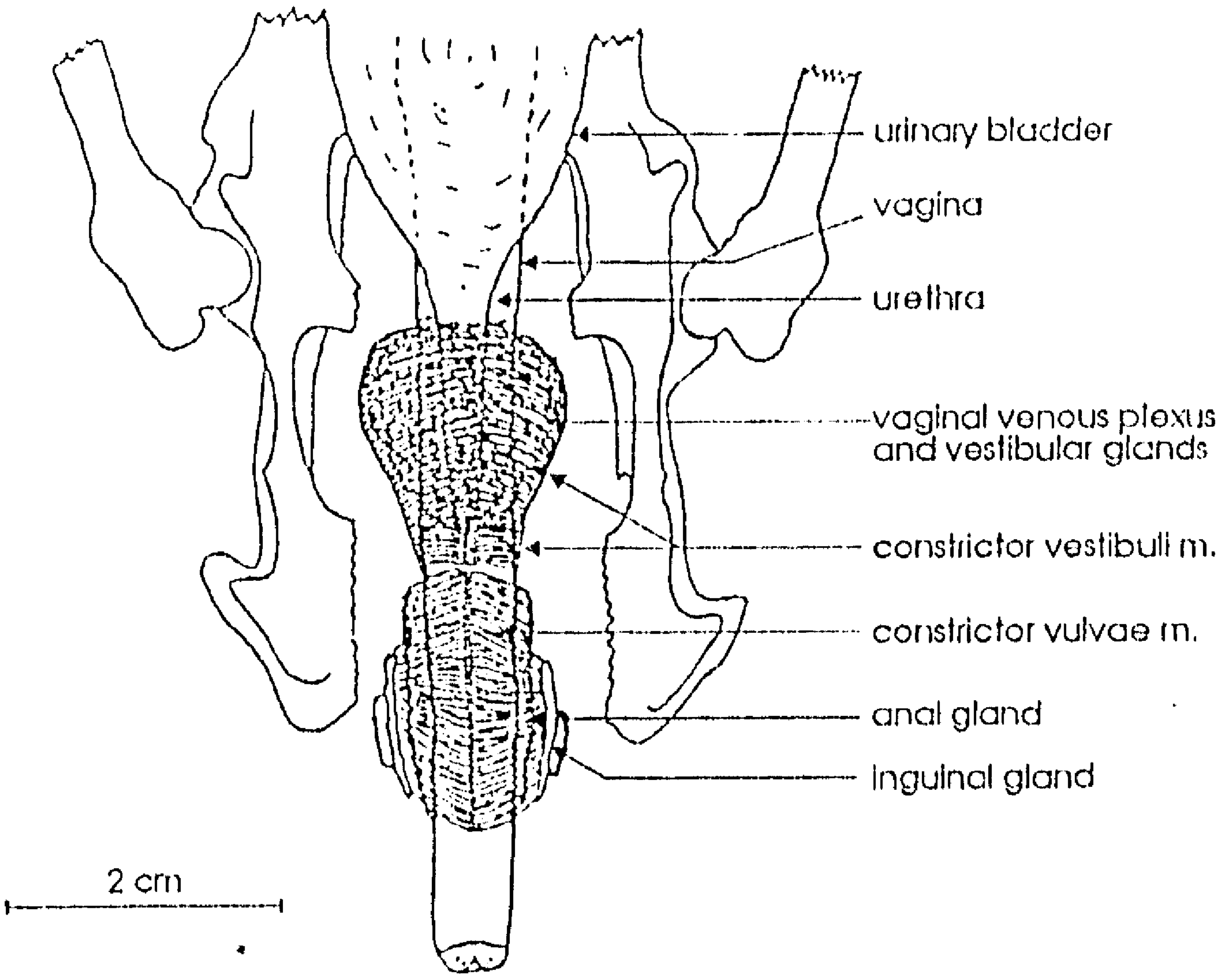

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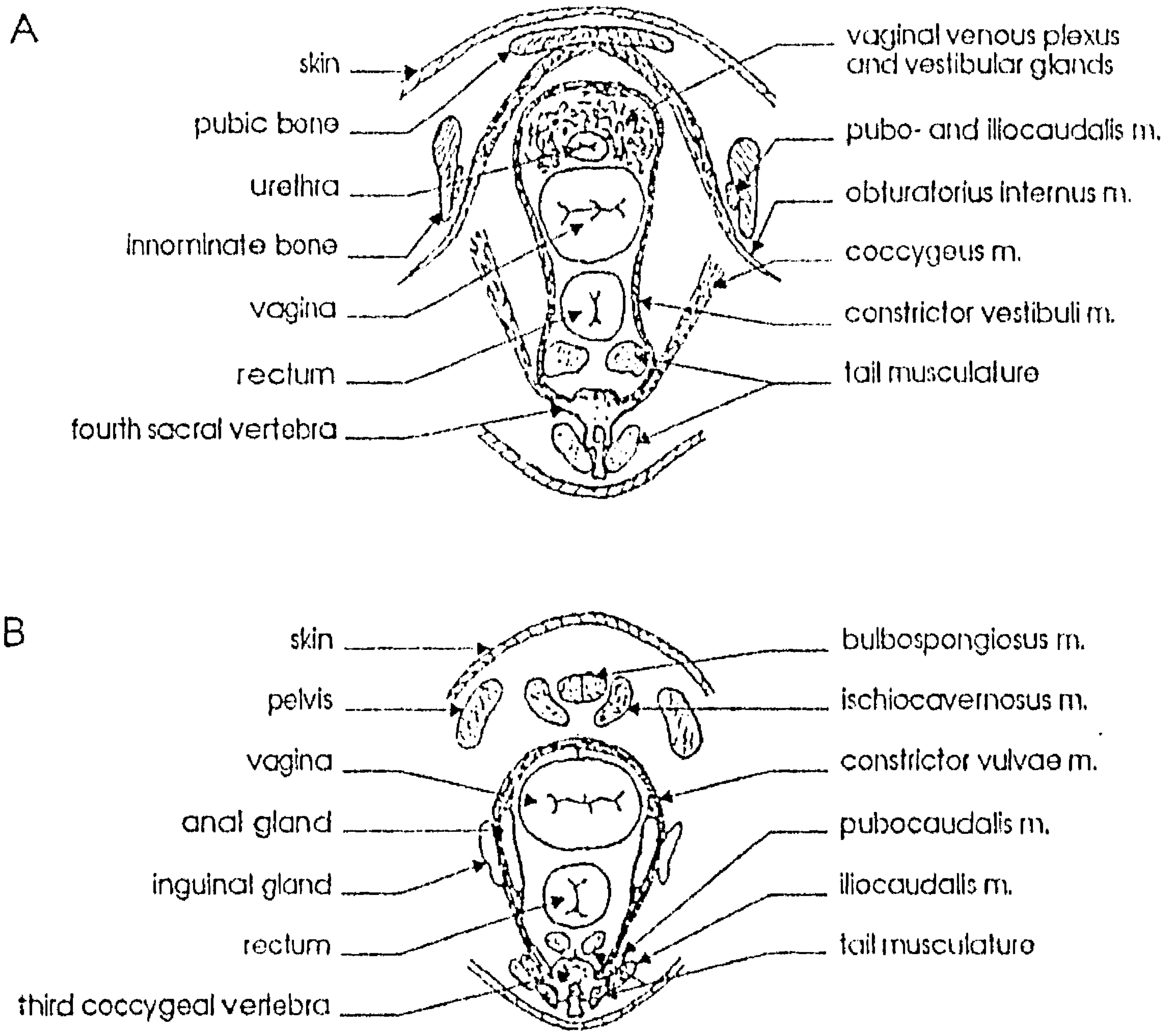

Fig. 5 (Martínez-Gómez et al.)

Fig. 6 (Martínez-Gómez et al.)

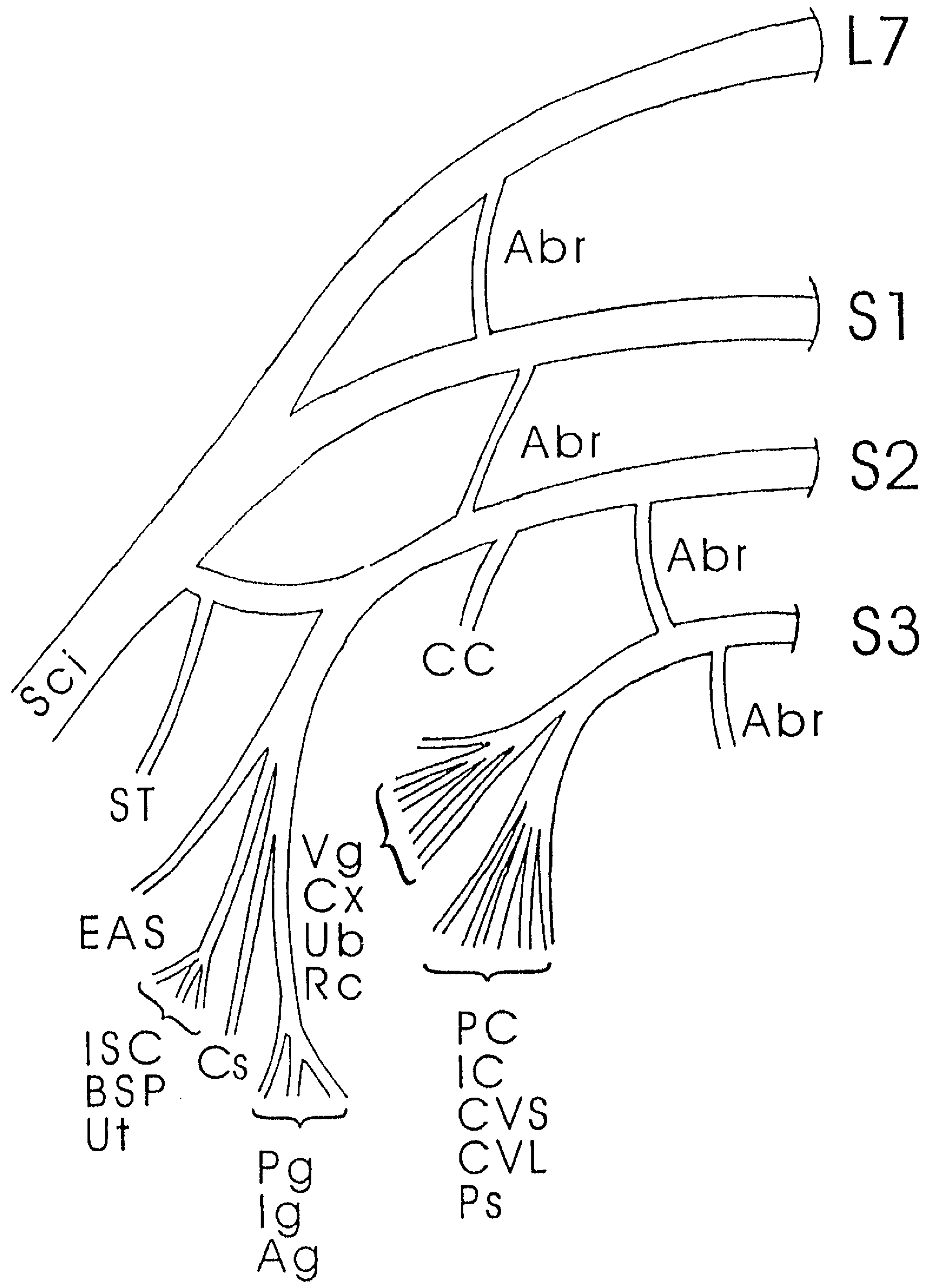

Fig. 7 (Martínez-Gómez et al.)