

## Facilitation at the sexually differentiated laryngeal synapse of *Xenopus laevis*

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**Abstract** Under physiological conditions, the laryngeal synapse of male *Xenopus laevis* exhibits marked facilitation during repetitive nerve stimulation. The male laryngeal synapse is weak and requires facilitation to produce muscle action potentials and ultimately sound. The female laryngeal synapse is strong; muscle contractions are produced to single nerve stimuli. We sought to determine if laryngeal synapses of males and females also differ in their ability to facilitate. To measure facilitation, laryngeal muscle action potentials were suppressed either postsynaptically by bathing the preparation in saline containing curare or presynaptically by bathing the preparation in reduced calcium/elevated magnesium saline. Facilitation of postsynaptic potential amplitude or quantal content in response to paired pulses was measured in male and female larynges: there is no sex difference in paired pulse facilitation. Facilitation in response to trains of stimuli, in curare-blocked preparations, increased and reached plateau values more rapidly in females than in males, although the facilitation between the last and first pulses in the train was the same in the sexes. Thus, the sexually differentiated behavior of this synapse is controlled more by a sex difference in synaptic strength than by a sex difference in the ability to facilitate.

**Key words** Synaptic efficacy · Paired pulse facilitation · Neuromuscular junction

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### Introduction

The sex-specific vocal behaviors of *Xenopus laevis* are produced by contraction of the intrinsic muscles of the larynx (Tobias and Kelley 1987). Sex differences in the laryngeal synapse itself modulate song and thus contribute to the sexual differentiation of this behavior. Synaptic strength, measured by quantal content, is lower at male than at female laryngeal synapses indicating less transmitter release (Tobias et al. 1995). In this study, we explore whether another form of synaptic plasticity, facilitation, also contributes to sex differences in laryngeal performance.

Under physiological conditions, male laryngeal fibers typically generate a subthreshold potential in response to a single nerve stimulus. At these synapses, trains of stimuli reveal facilitation of the postsynaptic response until threshold to generate an action potential is achieved (Tobias and Kelley 1988). Thus, males rely on facilitation to overcome the limitation of a weak synapse and produce sound. In contrast, female laryngeal muscle fibers typically generate an action potential in response to each nerve stimulus. Facilitation is not observed in female larynges since their predominantly strong synapses release suprathreshold levels of neurotransmitter (Tobias et al. 1995).

The goal of the present study was to determine whether facilitation is a sexually differentiated feature of the laryngeal synapse. To examine this question we measured facilitation at male and female synapses using paired pulses or trains of stimuli that mimicked essential features of the male song.

### Materials and methods

To examine facilitation, muscle action potentials must be blocked. Because comparing facilitation between the sexes necessitates comparing populations of fibers with inherently different properties, two complementary approaches were taken: action potentials were blocked both pre- and postsynaptically. Action potentials

were suppressed postsynaptically by adding curare to physiological saline to block the acetylcholine receptor and presynaptically by reducing calcium ion concentration to reduce neurotransmitter release. The latter approach was used previously to examine sex differences in synaptic strength (Tobias et al. 1995).

Two physiologically relevant stimulus paradigms were used to study facilitation: paired pulses and trains. Paired pulses were delivered at 15- and 30-ms interstimulus intervals (ISIs); these frequencies reflect the interclick intervals (ICIs) used in the attraction song produced by sexually mature males (Picker 1983; Wetzel and Kelley 1983). Paired pulses are a classic method for assaying facilitation and have the advantage of not producing action potentials (trains produce action potentials in reduced-calcium salines). Stimulus trains mimicked the fast trill portion of the male call: 15 ms ISI, 250 ms duration. Stimulus trains have the advantage of being a more physiological stimulus but could only be delivered to preparations bathed in saline containing curare.

#### Experimental preparation

Adult male and female *X. laevis* frogs were obtained from Nasco Co. (Ft. Atkinson, Wis., USA) and Xenopus Express (Beverly Hills, Fla., USA). Frogs were more than 2 years old (stage PM6 of Tobias et al. 1991), with body weights exceeding 25 g for males and 50 g for females. Frogs were housed in polycarbonate tanks containing dechlorinated, conditioned (Novaqua) tap water under a 12:12 (light:dark) cycle and fed frog brittle (Nasco) three times per week.

Frogs were deeply anesthetized by immersion in 0.1% MS-222 (ethyl *m*-amino benzoate methane sulfonic acid; Aldrich). Larynges were removed, pinned dorsal side up in a wax coated Petri dish and bathed in physiological saline containing (mmol·l<sup>-1</sup>): 116 NaCl, 2 KCl, 2.5 CaCl<sub>2</sub>, 3.0 MgCl<sub>2</sub>, 27.7 dextrose, 4 HEPES buffer. The viability of the preparation was tested by stimulating the motor nerve with 0.5-ms pulses of increasing voltage until the muscle visibly contracted.

#### Stimulation

Muscle fibers were impaled with glass microelectrodes (25–80 MΩ) filled with 0.1 mol·l<sup>-1</sup> KCl/3 mol·l<sup>-1</sup> KAc solution. Recordings were amplified (Getting Model 5A), digitized using a MacAdios II A/D converter and analyzed with the software Superscope IIe (GW Instruments, Cambridge, Mass., USA) on a Macintosh IIfx. To determine stimulus strength, the laryngeal nerve was stimulated with single 0.5-ms pulses of increasing voltage (up to 20 trials at each voltage) until a post-synaptic potential (PSP) was elicited in the muscle fiber; this voltage was then used in subsequent stimulation.

For paired pulses, each stimulus trial was separated from the next by 10 s. Approximately every third trial, the rate of stimulation (15 or 30 ms ISI) was alternated. The alternating pattern of stimulation (three trials at 15 ms ISI, three trials at 30 ms ISI, etc.) has a potential problem: three trials at one frequency might leave the cell in a state of depression or facilitation affecting subsequent trials. To test for this possibility we compared the quantal contents of the first pulses at each frequency (the response to the first pulse of any trial is presumably a control and should be uniform) in presynaptically blocked fibers. The quantal contents of responses to the first stimulus for the first 50 trials of stimulation at 15 ms ISI, and of the first 50 trials of stimulation at 30 ms ISI in each cell, were compared using a  $\chi^2$  test. There was no significant difference ( $P > 0.99$ ) between 15 ms (0.85 ± 0.76) and 30 ms ISI (0.84 ± 0.71). For stimulus trains delivered in salines containing curare, trains of 16 stimuli at 15 ms ISI were delivered to the larynx with a 90-s (four cases) or 3-min (nine cases) intertrial interval; there was no difference in facilitation between the two inter-trial intervals.

#### Facilitation indices of synapses in curare

To block muscle action potentials, curare (*α*-tubocurarine chloride; Sigma) was added to physiological saline and paired pulse facili-

tation was examined. Male larynges ( $n=7$ ) were bathed in 5  $\mu\text{mol}\cdot\text{l}^{-1}$  ( $n=1$ ), 6.25  $\mu\text{mol}\cdot\text{l}^{-1}$  ( $n=2$ ), 6.75  $\mu\text{mol}\cdot\text{l}^{-1}$  ( $n=1$ ), 7.5  $\mu\text{mol}\cdot\text{l}^{-1}$  ( $n=1$ ) and 10  $\mu\text{mol}\cdot\text{l}^{-1}$  ( $n=2$ ) curare. Female larynges ( $n=6$ ) were bathed in 10  $\mu\text{mol}\cdot\text{l}^{-1}$  ( $n=4$ ) and 20  $\mu\text{mol}\cdot\text{l}^{-1}$  ( $n=2$ ) curare. Recordings were obtained from only one muscle fiber per larynx. Facilitation in response to trains was studied in an additional six male and seven female muscle fibers. The preparation was bathed in a high concentration of curare (10–15  $\mu\text{mol}\cdot\text{l}^{-1}$ ) for 2–4 h, followed by a lower concentration (5–7.5  $\mu\text{mol}\cdot\text{l}^{-1}$ ) for the 30 min prior to recording; this protocol prevented muscle contractions in response to trains while maintaining sufficient postsynaptic sensitivity to observe PSPs. Recordings were obtained from two fibers each in male ( $n=3$ ) and female larynx ( $n=2$ ); in another female, three fibers were examined.

For paired pulse facilitation, a facilitation index ( $F_{IC}$ ) for each synapse was calculated as a ratio of the mean PSP amplitude of the second ( $A_2$ ) pulse to that of the first ( $A_1$ ) for each frequency:  $F_{IC} = A_2/A_1$ . The  $F_{IC}$ 's from male and female synapses, with an average of 70 trials per cell (range 32–92), were compared. For trains, a similar measure was derived except that the ratio obtained was the response to the last (16th) divided by the response to the first stimulus in a train; 4–15 trials per fiber.

#### Facilitation indices of synapses in reduced [Ca<sup>2+</sup>]<sub>o</sub>

Male ( $n=17$ ) and female ( $n=14$ ) larynges were bathed in salines with reduced [Ca<sup>2+</sup>]<sub>o</sub> and elevated [Mg<sup>2+</sup>]<sub>o</sub> (5 mmol·l<sup>-1</sup> in all experiments); osmoticity was maintained by changing NaCl concentration. Larynges were bathed in low [Ca<sup>2+</sup>]<sub>o</sub> saline until nerve stimulation failed to evoke muscle contractions (approximately 1 h). Recordings were obtained from a total of 21 male synapses (2 in 1.5 mmol·l<sup>-1</sup> [Ca<sup>2+</sup>]<sub>o</sub>, 18 in 1.0 mmol·l<sup>-1</sup> [Ca<sup>2+</sup>]<sub>o</sub>, and 1 in 0.5 mmol·l<sup>-1</sup> [Ca<sup>2+</sup>]<sub>o</sub>) and 15 female synapses (5 in 0.5 mmol·l<sup>-1</sup> [Ca<sup>2+</sup>]<sub>o</sub>, 5 in 0.375 mmol·l<sup>-1</sup> [Ca<sup>2+</sup>]<sub>o</sub>, and 5 in 0.25 mmol·l<sup>-1</sup> [Ca<sup>2+</sup>]<sub>o</sub>). Recordings were obtained from one or two muscle fibers per larynx with one exception; in one male larynx, recordings were obtained from four muscle fibers. The number of trials per fiber ranged from 63 to 586.

Facilitation in these fibers was measured by a change in quantal content between the first and second stimuli. Facilitation index was determined by calculating quantal contents ( $m = \ln(\text{total no. of trials}/\text{no. of failures})$ ; Del Castillo and Katz 1954) for the first and second pulse; facilitation index ( $F_{im}$ ) was calculated as the ratio of quantal contents to the first and second pulse:  $F_{im} = m_2/m_1$ .

Quantal size was determined for three synapses from which a large number of recordings (113, 222, and 586) at 15 ms ISI were obtained. Quantal size ( $q$ ) was calculated as  $q = V/m$ , where  $V$  represents the average PSP amplitude and  $m$  the calculated quantal content (Baxter et al. 1985). In calculations of the mean amplitudes, failures were included as zeros.

The large number of trials for these three synapses also enabled us to determine whether exhaustion was occurring during the experiment. Quantal content from the first 50 trials was compared to that from the last 50 trials (2.2 versus 2.6, 0.3 versus 0.2, and 1.4 versus 1.3, respectively); there was no significant difference between these values ( $P > 0.06$ ; Fisher test).

#### Statistical analyses

Because facilitation index is a ratio and can be ranked on an ordinal scale, nonparametric statistics were used for all comparisons. To determine whether facilitation indices are different in the sexes the median test was used (Siegel 1956). To examine sex differences in facilitation at the two stimulus frequencies, a Wilcoxon matched-pairs signed-rank test, which compares the same synapse at each frequency, and weights the magnitude of the difference, was used. Responses to trains of stimuli were fitted exponentially using Igor software on a Macintosh Power PC.

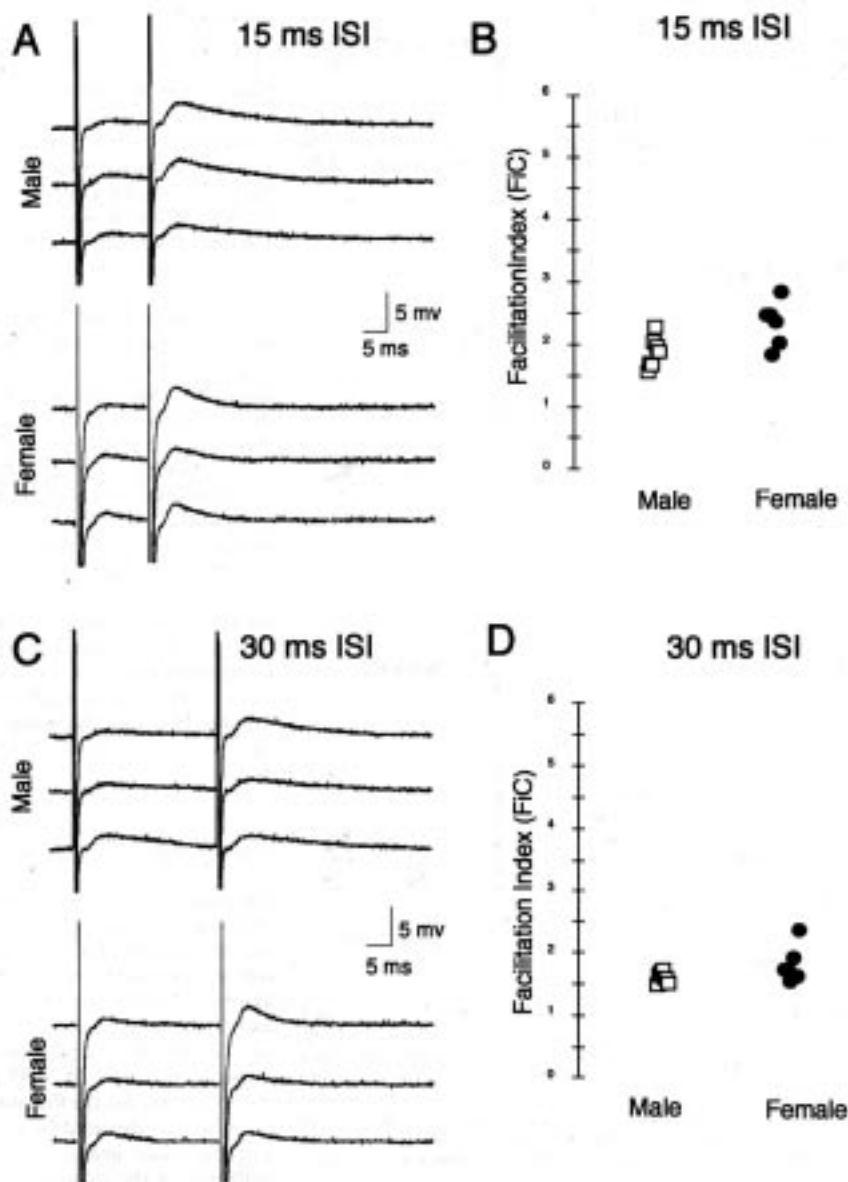
## Results

### Facilitation in response to paired pulses

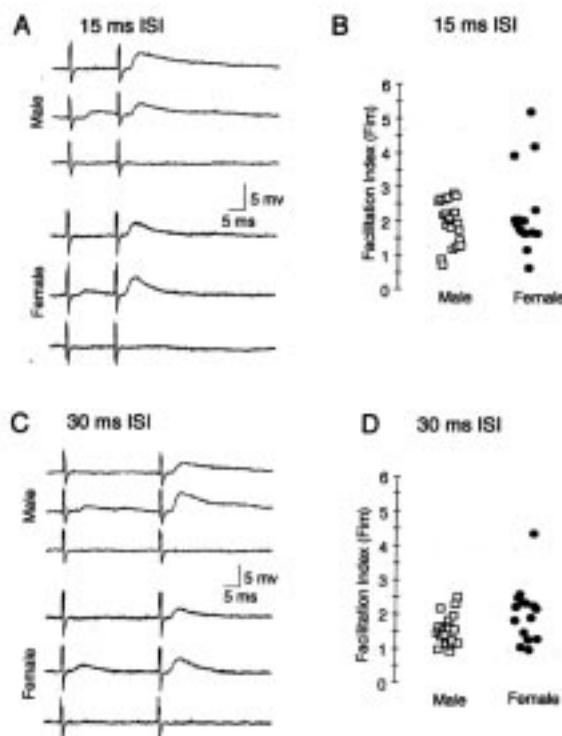
We first determined whether facilitation in response to paired pulses was different in the sexes. Responses to paired pulses from male and female synapses bathed in saline containing curare are illustrated (Fig. 1A, C); no failures were observed. The amplitude of the response following the second pulse was facilitated in nearly every

recording in both sexes at both frequencies of stimulation (first and second traces for each sex). In a few cases the response to the second pulse was similar in amplitude to the first (third trace; Fig. 1C). The mean  $F_{IC}$ 's for each synapse at 15 ms ISI (Fig. 1B) and 30 ms ISI (Fig. 1D) are shown; means for males and females overlap extensively. There is no sex difference in facilitation at 15 ms (males:  $1.9 \pm 0.3$ , females:  $2.3 \pm 0.4$ ;  $P > 0.07$ ) or 30 ms ISI (males:  $1.8 \pm 0.3$ , females:  $1.6 \pm 0.1$ ;  $P > 0.4$ ).

**Fig. 1A-D** Paired pulse facilitation in curarized synapses. **A** Three intracellular recordings from one male and one female laryngeal synapse stimulated at 15 ms interstimulus interval (ISI); postsynaptic potentials (PSPs), but not failures, are generated in response to all nerve stimulation. **B** Facilitation indices ( $F_{IC}$ ) from all synapses at 15 ms ISI; there is extensive overlap between the sexes. **C** Three intracellular recordings from one male and one female laryngeal synapse stimulated at 30 ms ISI. **D** Facilitation indices ( $F_{IC}$ ) from all synapses at 30 ms ISI; again, there is no difference between the sexes.



Facilitation in response to paired pulses was also measured in reduced calcium/elevated magnesium saline, the paradigm initially used to determine the sex difference in synaptic strength (Tobias et al. 1995). Sample recordings from male and female synapses stimulated at 15 ms (Fig. 2A) and 30 ms ISI (Fig. 2C) are illustrated. The typical response, for both sexes at both stimulus frequencies, was a failure to the first pulse and a PSP to the second pulse (top trace, Fig. 2A, C). Thus, quantal content increased in response to the second pulse; the synapse facilitated. Less frequently, PSPs (middle trace) or failures (bottom trace) occurred in response to both pulses. Facilitation indices ( $F_{im}$ ) were determined for all synapses at 15 ms ISI (Fig. 2B) and at 30 ms ISI (Fig. 2D). The mean  $F_{im}$  at 15 ms ISI is  $1.95 \pm 0.63$  for males and  $2.32 \pm 1.50$  for females. The mean  $F_{im}$  at 30 ms ISI is  $1.56 \pm 0.42$  for males and  $2.05 \pm 0.83$  for females. There is no sex difference in facilitation at 15 ms ( $P > 0.7$ ) or at 30 ms ISI ( $P > 0.2$ ).



**Fig. 2A–D** Paired pulse facilitation in reduced  $[Ca^{2+}]_i$ -elevated  $[Mg^{2+}]_o$ . **A** Three intracellular recordings from one male and one female synapse at 15 ms ISI. Both PSPs and failures were seen following the first or second stimulus. **B** Facilitation indices ( $F_{im}$ ) from all synapses stimulated at 15 ms ISI; there is extensive overlap between the sexes. **C** Three intracellular recordings from one male and one female synapse at 30 ms ISI. **D** Facilitation indices ( $F_{im}$ ) from all synapses at 30 ms ISI again demonstrate considerable overlap between the sexes.

The facilitation indices measured under these conditions are nearly identical to those achieved in postsynaptically blocked fibers.

To determine whether facilitation differed between the two frequencies of stimulation, facilitation indices for each synapse at the two frequencies were compared in males and females. Facilitation in postsynaptically blocked fibers was significantly greater at 15 ms than at 30 ms ISI in both sexes (males:  $P = 0.03$ , females:  $P = 0.04$ ). In presynaptically blocked fibers, there is a significant difference in facilitation indices between the two stimulus frequencies in males ( $P = 0.04$ ) but not in females ( $P = 0.67$ ).

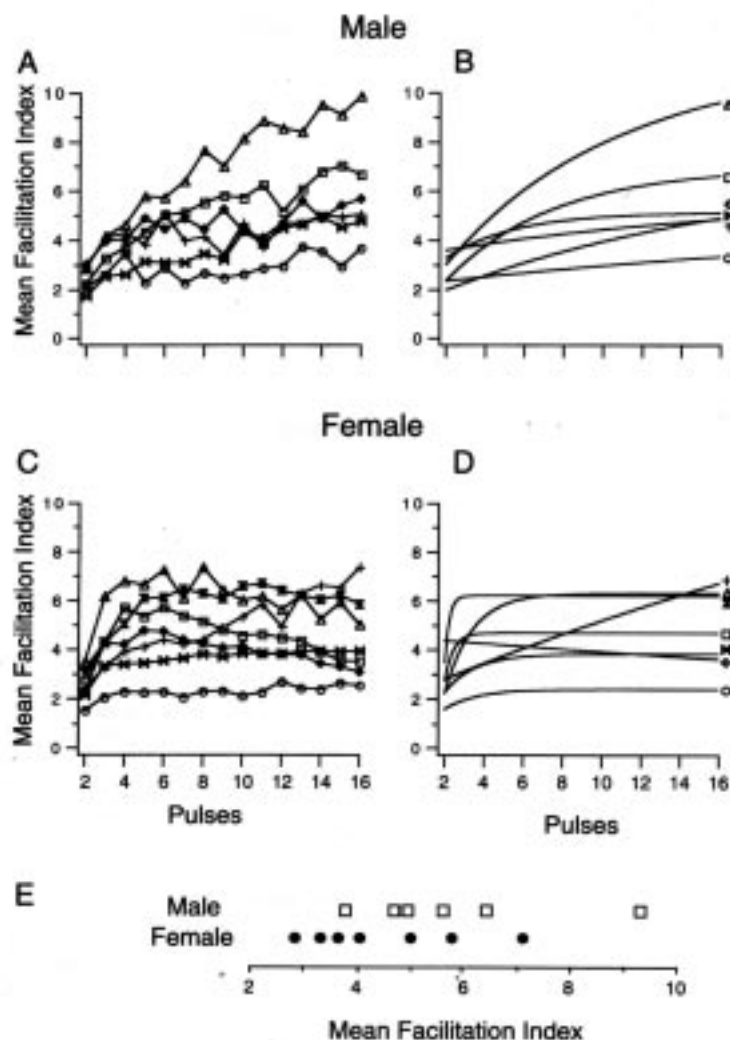
#### Facilitation in response to stimulus trains

Since there was no sex difference in facilitation in response to paired pulses, we next considered the possibility that there might be a sex difference in response to a longer and more physiological stimulus. Male laryngeal synapses facilitate, in physiological saline, in response to stimulus trains which mimic the fast portion of the mate call; a trill lasting ~250 ms with an interclick interval of ~15 ms (Tobias and Kelley 1988). Facilitation indices at male and female synapses in salines containing curare in response to stimulus trains are shown in Fig. 3. Facilitation between the first and each succeeding pulse in the train is shown for male (Fig. 3A) and female (Fig. 3C) synapses. Each point represents the mean facilitation index (number of trials = 4–15 for each fiber). There is no significant sex difference in the facilitation index comparing the first and last PSPs in the train (males =  $5.9 \pm 2.2$ , females =  $4.5 \pm 1.7$ ;  $P = 0.18$ ; see Fig. 3E). However, male synapses continue to facilitate somewhat throughout the stimulus train while female synapses tend to plateau. The data in Fig. 3A and C can be fitted to a single exponential function (Fig. 3B, D, respectively). In females, five of the seven records reveal an early increase in facilitation which reaches a plateau rapidly. In males, the initial increase in facilitation is not as steep as in females and only one record reveals a plateau.

#### Quantal size

Facilitation typically affects quantal content without affecting quantal size. Recording from fibers in reduced calcium permitted measurement of quantal size. Quantal size was calculated for three synapses from which a large number of trials at 15 ms ISI were obtained (male: 113 and 586 trials; female: 222 trials). The ratios of quantal size for the first and second pulses for each synapse respectively were 1.06, 0.97 and 1.04. Thus, quantal size is very similar for the first and second pulse. Quantal size can also be estimated by examining the peaks in a PSP amplitude histogram; the peaks should occur at integer multiples of the quantal size. For this purpose, we an-

**Fig. 3A-E** Facilitation to stimulus trains in curarized synapses. The mean facilitation index between the first and each succeeding pulse in a stimulus train for all male (A) and female (C) synapses. Each point represents the mean of 4–15 trials. Data in A and C can be fitted to a single exponential function (B and D, respectively). E Facilitation indices, measured as the ratio between the first and last (16th) pulses in a train, for all male and female synapses. There is complete overlap in facilitation indices between the sexes.



alyzed data from the synapse with the most recordings (586; quantal content is 0.29). The PSP amplitudes of the response to the first pulse are distributed around a single value (2 mV; Fig. 4A), which in theory corresponds to one quantum. This value is in close agreement with the quantal size calculated for this cell (1.8 mV). For the second pulse, PSP amplitudes again have a modal value of 2 mV (Fig. 4B). There are fewer failures to the second pulse than the first; the distribution of amplitude values is suggestive of another peak at 3.5 mV which could correspond to twice the quantal value (3.6 mV). The quantal values reported here agree with miniature end-plate potential amplitudes measured in a previous study (males =  $1.1 \pm 0.6$ , females =  $1.8 \pm 1.2$ ; Tobias et al. 1995).

## Discussion

The isolated larynx of *X. laevis* provides the behavioral neurophysiologist with an opportunity to relate synaptic properties to the production of vocal behaviors. We have previously shown that this preparation can, when both laryngeal nerves are stimulated at appropriate rates, produce click trains which closely resemble actual *X. laevis* vocalizations (Tobias and Kelley 1987). To what extent do properties of male and female vocal synapses contribute to the sexually dimorphic physiology of the vocal organ? Under physiological conditions the majority of male laryngeal synapses produce sub-threshold potentials in response to nerve stimulation while the majority of female laryngeal synapses produce action potentials (Tobias and Kelley 1988). The results

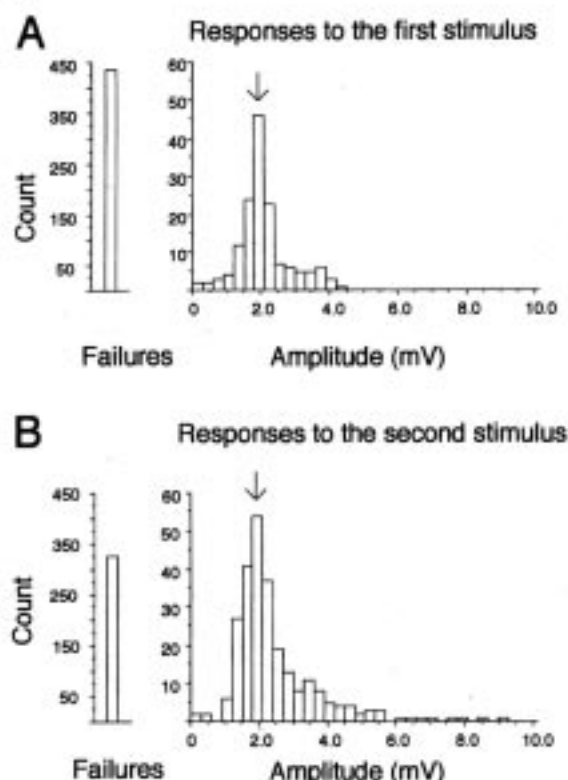


Fig. 4A, B Quantal size of the first and second responses to paired pulse stimuli. PSP amplitude histograms for responses to the first (A) or second (B) stimulus in a pair. For both distributions, the peak modal amplitude is approximately 2.0 mV. The quantal size calculated for the cell was 1.8 mV. The number of failures to the first and second stimuli are also shown

of a quantal analysis in adult larynx strongly suggest that the weakness of the male synapse is presynaptic in origin; quantal content is significantly higher at female than at male synapses, while there is no sex difference in miniature endplate potential amplitudes (Tobias et al. 1995).

During the rapid trills of male song the laryngeal synapse is repetitively excited producing facilitation and consequent muscle action potentials and sounds. Female vocal synapses do not require facilitation to produce sounds because the synapse is strong enough that each nerve impulse produces a muscle action potential. Because facilitation is observed under physiological conditions only in males – where it is required for song production – we wished to know if the ability to facilitate differs in the sexes. Facilitation was measured by blocking muscle action potentials and repeatedly stimulating the laryngeal nerve. Whether the blockade was accomplished postsynaptically – with curare – or presynaptically – by lowering calcium and raising magnesium external concentrations – no sex difference in

facilitation index was apparent. We conclude that sex differences in vocal performance are primarily related to the sex differences in synaptic strength we have previously described (Tobias and Kelley 1988; Tobias et al. 1995).

While the laryngeal synapse is a powerful behavioral preparation, it has a number of limitations for neurophysiological studies. First, the profound sex difference in synaptic strength means that measurements requiring equivalent reductions in synaptic efficacy cannot be carried out under the same conditions in males and females. For example, in a previous study (Tobias et al. 1995) we determined that considerably more stringent ion conditions are required to block muscle contractions in female ( $0.5 \text{ mmol} \cdot \text{l}^{-1} \text{ CaCl}_2$ ,  $5.0 \text{ mmol} \cdot \text{l}^{-1} \text{ MgCl}_2$ ) than in male ( $1.5 \text{ mmol} \cdot \text{l}^{-1} \text{ CaCl}_2$ ,  $4.0 \text{ mmol} \cdot \text{l}^{-1} \text{ MgCl}_2$ ) larynges. In the present study, we wished to measure facilitation in the sexes from comparable starting points of evoked release. External ion concentrations were altered until quantal contents were similar in males and females; no sex difference in facilitation was observed under these conditions. One interpretation of these results is that the lower concentrations of calcium required to block action potentials in females increased facilitation to the point where it matched male levels. While the failure to observe sex differences in facilitation with postsynaptic blockade argues against this interpretation, it remains a formal possibility.

Another disadvantage of the larynx is the thick bipennate muscle which slows diffusion into the muscle and prevents visualization of the synapse during recordings. Is it possible that a systematic bias toward one sex in the distance of the electrode from the synapse accounts for a failure to observe a sex difference in facilitation? For saline containing curare, this presents less of a problem since no failures are observed and measurements of facilitation are relative. However, in reduced-calcium salines (using the method of failures to measure quantal content), if the electrode were always located further away from the synapse in one sex, the frequency with which synaptic failures occur in response to the initial stimulus might be over estimated. To examine this possibility we have measured the amplitudes and rise times of miniature endplate potentials (MEPPs) in both sexes (Tobias et al. 1995). MEPP frequency in the larynx is extremely low and can only be measured when external calcium concentration ( $15 \text{ mmol} \cdot \text{l}^{-1}$ ) is high; MEPP frequency under these conditions is 0.3 Hz. Neither MEPP amplitude nor rise time, nor the input resistance of muscle fibers, differ for males and females, arguing against a systematic bias in favor of one sex in our ability to detect synaptic events. While MEPPs were rarely observed using the lower calcium salines required to study facilitation, the conditions of recording used here were identical to those of the previous study and it is thus unlikely that distance from the synapse was systematically biased by sex in this study.

Under physiological conditions three types of synapses can be distinguished in male and female larynges

(Tobias and Kelley 1988): type I synapses that produce either a subthreshold PSP or an action potential depending on stimulus strength; type II synapses that produce only a subthreshold PSP to a single nerve stimulus regardless of strength; and type III synapses that always produce an action potential provided stimulus strength is above threshold. The distribution of synapse types differs markedly in the sexes: type II is the predominant type in males (75%) and type III is the predominant type in females (85%); type I is rare in females and more common in males (18%). Could facilitation differ between the sexes in a particular fiber type? Because type II synapses predominate in males and type III synapses in females, these are also likely to dominate our recordings. If so, facilitation is comparable for these sex-typical fiber types. However, the low frequency of other fiber types in each sex could mask a fiber-specific difference in facilitation in the population of fibers examined in this study. To determine fiber type and measure facilitation in the same muscle fiber requires maintaining an intracellular recording while changing from physiological saline to a saline which blocks action potentials. The very small diameter of laryngeal muscle fibers (Sassoon et al. 1987) and the time required for equilibration of a new saline solution (>1 h) hamper the success of this experiment. In addition, muscle fiber type identification elicits contractions, further increasing the probability of dislodging the electrode.

Characteristic features of facilitation at the male synapse may contribute to male-specific song. We show here that male synapses demonstrate increasing PSP facilitation throughout stimulus trains. The male courtship song is composed of a fast trill (15 ms ICI) in which successive clicks become progressively louder followed by a slower trill (30 ms ICI) in which the amplitude is either constant or fluctuates non-uniformly (Wetzel and Kelley 1983). When the male synapse is provided with stimulation mimicking the fast trill portion of the song, facilitation increases throughout the trill as does sound amplitude. The prolonged increase in facilitation guarantees that even the weakest fibers will be recruited to produce action potentials, and contract, during the male trill. In addition, facilitation is stronger at 15 than at 30 ms ISI indicating that laryngeal synapses can distinguish these relatively small, but behaviorally relevant, differences in frequency; only the 15-ms ICI trill is amplitude modulated. Amplitude modulation is a distinguishing feature of male songs in *X. laevis* and is not present in related species (Vigny 1979). Differences in amplitude modulation, having as a proximate cause the facilitation of weak laryngeal synapses in males, could contribute to the isolation of sympatric populations and thus to speciation.

Our results demonstrate that despite a large sex difference in synaptic strength there is no sex difference in facilitation suggesting that the mechanisms modulating release and facilitated release may be distinct. For example, lowering the temperature to 0 °C elicits a delay in

maximal facilitation without a delay in release (Yamada and Zucker 1992; Kamiya and Zucker 1994; Van der Kloot 1994). If release and facilitated release are distinct, facilitation could be similar at strong and weak synapses. In *Rana pipiens* the fast component of facilitation was examined in proximal (strong) and distal (weak) portions of the same cutaneous pectoris neuromuscular junction with no significant difference in facilitation (Robitaille and Tremblay 1991). In mouse diaphragm, facilitation was examined over a range of quantal contents similar to that used in this study (0.1–3.0); again, no correlation between strength and facilitation was found (Bain and Quastel 1992). A variation in facilitation without a variation in strength has been demonstrated in another androgen-sensitive muscle of *X. laevis*: in the flexor carpi radialis, testosterone enhances facilitation without affecting release to a single stimulus (Nagaya and Herrera 1995). Mallart and Martin's (1968) study of frog sartorius showed that there was some dependence of facilitation on quantal content but only for the weakest synapses. In general, whether release varies endogenously – by sex, hormonal treatment or within a single synapse – or is induced to vary exogenously – by depressing synaptic efficacy – the release process and the phenomenon of facilitated release appear to be independent.

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## References

- Bain AI, Quastel DMJ (1992) Multiplicative and additive  $Ca^{2+}$  dependent components of facilitation at mouse endplates. *J Physiol (Lond)* 455: 383–405
- Baxter DA, Bittner GD, Brown TH (1985) Quantal mechanism of long-term synaptic potentiation. *Proc Natl Acad Sci USA* 82: 5978–5982
- Del Castillo J, Katz B (1954) Quantal components of the end-plate potential. *J Physiol (Lond)* 124: 560–573
- Kamiya H, Zucker RS (1994) Residual  $Ca^{2+}$  and short term synaptic plasticity. *Nature* 371: 603–606
- Mallart A, Martin AR (1968) The relation between quantum content and facilitation at the neuromuscular junction of the frog. *J Physiol (Lond)* 196: 593–604
- Nagaya N, Herrera AA (1995) Effects of testosterone on synaptic efficacy at neuromuscular junctions in a sexually dimorphic muscle of male frogs. *J Physiol (Lond)* 483: 141–153
- Picker MD (1983) Hormonal induction of the aquatic phonotactic response of *Xenopus*. *Behaviour* 84: 74–80
- Robitaille R, Tremblay JP (1991) Non-uniform responses to  $Ca^{2+}$  along the neuromuscular junction: effects on the probability of spontaneous and evoked transmitter release. *Neuroscience* 40: 571–585
- Sassoon D, Gray G, Kelley D (1987) Androgen regulation of muscle fiber type in the sexually dimorphic larynx of *Xenopus laevis*. *J Neurosci* 7: 3198–3206
- Siegel S (1956) Nonparametric statistics for the behavioral sciences. McGraw-Hill, New York

- Tobias ML, Kelley D (1987) Vocalizations of a sexually dimorphic isolated larynx: peripheral constraints on behavioral expression. *J Neurosci* 7: 3191-3197
- Tobias ML, Kelley D (1988) Electrophysiology and dye-coupling are sexually dimorphic characteristics of individual laryngeal muscle fibers in *Xenopus laevis*. *J Neurosci* 8: 2422-2429
- Tobias ML, Marin M, Kelley DB (1991) Development of functional sex differences in the larynx of *Xenopus laevis*. *Dev Biol* 147: 251-259
- Tobias ML, Kelley DB, Ellisman M (1995) A sex difference in synaptic efficacy at the laryngeal neuromuscular junction of *Xenopus laevis*. *J Neurosci* 15: 1660-1668
- Van der Kloot W (1994) Facilitation of transmission at the frog neuromuscular junction at 0 degrees C is not maximal at time zero. *J Neurosci* 14: 5722-5724
- Vigny C (1979) The mating calls of 12 species and sub-species of the genus *Xenopus* (Amphibia: Anura). *J Zool (Lond)* 188: 103-122
- Wetzel DM, Kelley DB (1983) Androgen and gonadotropin effects on male mate calls in South African clawed frogs, *Xenopus laevis*. *Horm Behav* 17: 388-404
- Yamada WM, Zucker RS (1992) Time course of transmitter release calculated from simulations of a calcium diffusion model. *Biophys J* 61: 671-682