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Gating Kinetics of Nonjunctional Acetylcholine Receptor Channels in Developing *Xenopus* Muscle[1]

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Abstract

The development of nonjunctional acetylcholine (ACh) receptor gating kinetics was studied in myotomal muscle of *Xenopus laevis* by spectral analysis of ACh-induced noise. The results are consistent with the idea that there is a developmental shift in relative numbers of receptors with fast and slow gating kinetics. For about the first 20 hr following the onset of ACh sensitivity, the majority of channel openings contributing to the ACh noise were slow, having an apparent mean open time of about 3 msec. Shortly before the time of hatching, there began to appear a significant contribution to the noise from channels having a mean open time of less than 1 msec. The relative amount of fast channel activity increased during the 2 days following hatching, and by the age of maturity of the myotomal synapse, most of the ACh noise arose from fast channel activity. A comparison of nonjunctional kinetics with synaptic currents at maturity suggests that the control of channel gating time is similar at junctional and nonjunctional regions.

Two types of acetylcholine (ACh) receptor channels, differing in their gating kinetics, have been observed in developing mammalian and amphibian skeletal muscle. One type, appearing early in development, is characterized by long apparent open times; it is followed later by the appearance of a second type with faster gating kinetics. In rats, open times were reported to be 3 to 4 msec in neonatal muscle and less than 1 msec in mature muscle, whereas a mixture of the two classes of receptors was found at intermediate stages of development (Sakmann and Brenner, 1978; Fischbach and Schuetze, 1980; Michler and Sakmann, 1980). A similar transition from slow to fast gating kinetics was observed at ectopic synapses formed on denervated muscle in rats (Brenner and Sakmann, 1983). In a study of embryonic muscle of *Xenopus laevis*, the ACh receptors were found to have apparent open times which declined from 2 to 4 msec to about 1 msec during the first 3 days of development, according to spectra of ACh noise (Kullberg et al., 1981). It was not clear from that study whether the change in apparent open time was due to a shift in relative numbers of slow and fast channels, as found in rat muscle, or to a gradual change in the gating time of all of the channels. The first possibility is supported by single-channel recordings from *Xenopus* muscle *in vitro*, which revealed two classes of ACh receptors with slow and fast gating times (Brehm et al., 1983). The relative frequency of fast channel openings was found to increase with age of the cultures. Single-channel recordings from mature *Xenopus* muscle *in vivo* also demonstrated the existence of discrete classes of channels with slow and fast gating kinetics (Brehm et al., 1984).

The objectives of the present study were to describe the apparent open times of nonjunctional ACh receptors in *Xenopus* myotomal muscle *in vivo*, from the time of their earliest detectable presence on the membrane to the time of maturity of the synapse, by means of extracellular noise recording. We have confined our noise recordings to nonjunctional regions to avoid possible overlap of junctional and nonjunctional channel activity. In light of the findings that mammalian ACh receptors undergo discrete transitions from slow to fast kinetics and that *Xenopus* muscle can produce two kinds of channels, we paid particular attention to the question of whether the developmental change in ACh receptor open time can be described adequately as a shift between relative numbers of two classes of ACh receptors.

Materials and Methods

Experiments were performed on embryos and tadpoles of *Xenopus laevis* which were staged according to the criteria of Nieuwkoop and Faber (1967). Details of rearing embryos and tadpoles were similar to those described previously (Kullberg et al., 1977). All recordings were obtained from myotomal muscle cells in the anterior third of the tail. The muscle was pinned to a Sylgard-coated dish and bathed in Ringer containing (in millimolar concentration): NaCl, 110; KCl, 3; $CaCl_2$, 1.8; and HEPES-NaOH buffer, 8 (pH 7.4). Experiments were done at room temperature (21° to 24°C).

Recordings of ACh-induced noise were made with extracellular electrodes containing dilute solutions of ACh, in a manner similar to that described by Schuetze et al. (1978). With this technique, the recorded signal is a fluctuating extracellular voltage which corresponds to the ACh-induced membrane current noise. Electrode inner tip diameters were 3 to 6 μm and resistances were about 2 to 4 megohms. In most experiments, the concentration of ACh ranged from 2 to 5 μM, and a slight positive pressure (5 to 10 cm of H_2O) was applied to the electrode. By gently pressing the electrode tip against nonjunctional regions of membrane, it was often possible to obtain stable records of ACh-induced noise lasting a minute or longer. Pressure of the electrode against the muscle membrane produced a seal resistance of 1 to 3 megohms and caused a corresponding increase in resistance noise. Control noise records were taken by pressing the electrode against non-muscle tissue, either notochord or epidermis, so that the same seal resistance was achieved. In some of the earlier experiments, the ACh concentration in the pipette was 20 to 30 μM and positive pressure was not applied to the electrode. Under these conditions, the agonist concentration at the tip was rapidly diluted, and the pipettes often became unusable after several minutes. These recordings gave results similar to those with lower agonist concentrations; however, the method was not preferred because of the lack of control of agonist concentration.

Noise recordings were sampled at 0.49-msec intervals and spectra were computed from 512 data points, giving a record length of about 250 msec, a bandwidth resolution of 4 Hz, and a Nyquist frequency of 1020 Hz. Noise records were low pass filtered at 800 Hz (24 dB/octave rolloff). In order to obtain high resolution spectra suitable for curve fitting, up to 500 individual spectra were averaged from a single recording, requiring total record lengths

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of up to 125 sec. Control spectra of resistance and instrumentation noise were subtracted from the spectra obtained in the presence of ACh. The resulting difference spectra were normalized to the zero frequency asymptote and displayed on a log-log scale. The log-transformed difference spectra were fitted by weighted, nonlinear least squares with both single and double Lorentzian theoretical curves. The spectral points were fitted out to 5% of the zero frequency asymptote, up to a maximum of 600 Hz. Because the density of spectral estimates increases with frequency when the data are displayed logarithmically, we chose a weighting which was inversely proportional to frequency. With such weighting, each logarithmic unit of the spectrum on the frequency axis has equal importance in determining the best fit; otherwise the fit would be dominated by the high frequency power. The curve fitting was carried out by iterative variation of parameter values in a search for the least sum of squared deviations. For single Lorentzian curves, the fitting parameters were the zero frequency asymptote, $S(0)$, and the corner (half-power) frequency f_c, as used in the following equation:

$$S(f) = S(0)/(1 + (f/f_c)^2)$$

For double Lorentzian curves, the fitting parameters were the corner frequencies of the slow and fast components, f_{cs} and f_{cf}, and the sum and ratio of their zero frequency asymptotes, $S(0)_s$ and $S(0)_f$, as they appear in the double Lorentzian equation:

$$S(f) = S(0)_s/(1 + (f/f_{cs})^2) + S(0)_f/(1 + (f/f_{cf})^2)$$

In the double Lorentzian fits, it was found that the best-fitting parameters were quite dependent on the starting point of the search. For instance, when starting from the best fit by eye, it was possible on subsequent trials to have variations of more than 20% in the value of estimated parameters. To minimize biases resulting from the starting values, a set of more than 9000 theoretical curves was searched to find the best initial fit for each spectrum; the resulting parameter values were then iteratively varied by small increments to find the local least sum of squared deviations.

Results

Recordings of ACh-induced noise were made at nonjunctional regions of myotomal muscle in *Xenopus* embryos and tadpoles ranging in age from stage 20 (21¾ hr) to stage 50 (15 days). Previous work has shown that ACh sensitivity first appears at about stage 19/20 and, by 2 weeks later, the myotomal neuromuscular junction is mature (Blackshaw and Warner, 1976; Kullberg et al., 1977, 1980). Throughout this age span, the density of nonjunctional ACh receptors was high enough to permit recording of channel noise by use of ACh-filled extracellular electrodes. The distribution of receptors in nonjunctional regions did not appear to be uniform, since the ACh-induced noise signal varied in amplitude and occasionally was absent. Pressing the recording electrode against nonmuscle tissue, such as notochord or epidermis, resulted in an increase in resistance noise, but no noise with the same spectral density as seen in recordings from muscle. The spectra of resistance noise were flat between zero frequency and the cutoff frequency of the recording filter.

The ease of recording noise varied with the stage of development, presumably reflecting the density of ACh receptors and their gating kinetics. Regions of high density were quite sparse in stages 20 and 21 and gradually became more common. The frequency of successful recordings was greatest between stages 30 and 40, after which it became progressively more difficult to find spots with enough activity to compute spectra suitable for analysis. A gradual reduction in nonjunctional receptors following stage 36 has been reported by Chow and Cohen (1983). Compounding the problem of reduced numbers of receptors is the fact that the briefer open times of channels present at later stages should result in a reduced amplitude of the noise (Anderson and Stevens, 1973).

We were interested in determining whether one or two classes of ACh receptors were present on the developing muscle membrane. We therefore fitted all spectra with double- and single-component Lorentzian curves by least squares. Representative spectra from different developmental stages are shown in Figure 1. The double and single Lorentzian curves appear to fit the data equally well at an early stage (20), whereas at intermediate stages of development (stages 35 and 43) there is an obvious inadequacy in the single Lorentzian fit. At a late stage (50), the double Lorentzian curve fits the data slightly better than the single curve.

As a check on this visual impression, we compared the goodness of fit of double versus single Lorentzian curves by computing the ratios (double/single) of their residual sums of squares (SSQs). A ratio of 1 indicates equivalent goodness of fit, whereas higher ratios indicate comparatively better fits by double Lorentzian curves. As expected, the SSQ ratios were greatest at intermediate stages of development (stages 35 to 43), when the single Lorentzian spectra appeared to fit most poorly. During this period, the mean SSQ ratio was 3.9 ± 2.1 (n = 28). By comparison, the lowest ratios occurred at early stages (20 to 24), where more than half of the SSQ ratios were less than 1.1 and the average value was 1.2 ± 0.4 (n = 24). At late stages (45 to 50), the SSQ ratios had a slightly higher mean of 1.6 ± 0.6 (n = 13).

Each spectrum, when fitted by a double Lorentzian curve, yielded two estimates of corner frequencies, referred to here as high and low corner frequencies. From stage 20 to stage 30, the majority of the high corner frequencies fell between 30 and 100 Hz (Fig. 2*A*). Of 33 spectra at those stages, only 3 had high corner frequencies exceeding 100 Hz. In contrast to the estimates from early stages, the high corner frequencies of spectra from stage 35 and later were invariably greater than 100 Hz and ranged up to 370 Hz. There appeared to be a transitional period at about stage 33/34 when the fast components of the spectra began to achieve higher values. A smaller increase in the low corner frequency took place at the same time (Fig. 2*B*). The low corner frequencies ranged from 15 to 50 Hz at stages 20 through 30, and from 20 to 160 at stages 35 through 50.

The data in Figure 2 imply that there is a change in spectral shape during development which occurs most rapidly at about stage 35, shortly before the time of hatching (stage 37). The superior fit of double Lorentzian curves to the spectra at intermediate and late stages of development favors the idea of two channel types in developing muscle as opposed to a single type. Because of the wide scatter of high and low corner frequencies, it is difficult to make a clear distinction between the different channel types. However, on the basis of the data presented in Figure 2*A*, the best choice for a dividing point appears to be about 100 Hz. All of the high corner frequencies beyond stage 35 exceeded 100 Hz, and all of those from stages less than 30, with a few exceptions, were less than 100 Hz. We propose, therefore, that the Lorentzian components with corner frequencies greater than 100 Hz result from the activity of channels which are kinetically distinct from those initially present on the membrane.

Whereas the majority of spectra at intermediate and late stages were clearly fitted better by double Lorentzian curves, there were numerous spectra at early stages which appeared to be adequately described by single Lorentzian curves. In these cases, the high and low corner frequencies were both slow (≤100 Hz), the SSQ ratios were close to unity, and the goodness of fit of single versus double Lorentzian curves could not be discriminated by eye. There were also a few spectra at late stages which appeared to be adequately fitted by single Lorentzian curves. In these spectra, the double Lorentzian corner frequencies were both fast (>100 Hz). It appears that the best description of the change in channel gating kinetics is given in terms of both single and double Lorentzian data, as done in Figure 3. There, we have plotted the single Lorentzian corner frequencies of spectra which appeared to arise from only one class of channels; otherwise, we have plotted the double Lorentzian corner frequencies. The spectra were predominantly single Lorentzian from stage 20 to 33/34, and double thereafter.

According to the data in Figure 3, the average corner frequency of the slow channels throughout development was 49 ± 17 Hz (n = 85), and that of the fast channels was 182 ± 65 Hz (n = 48). To express the spectral data in terms of channel gating kinetics, the mean corner frequency of each class of channels was used to calculate a corresponding apparent mean channel open time (τ),

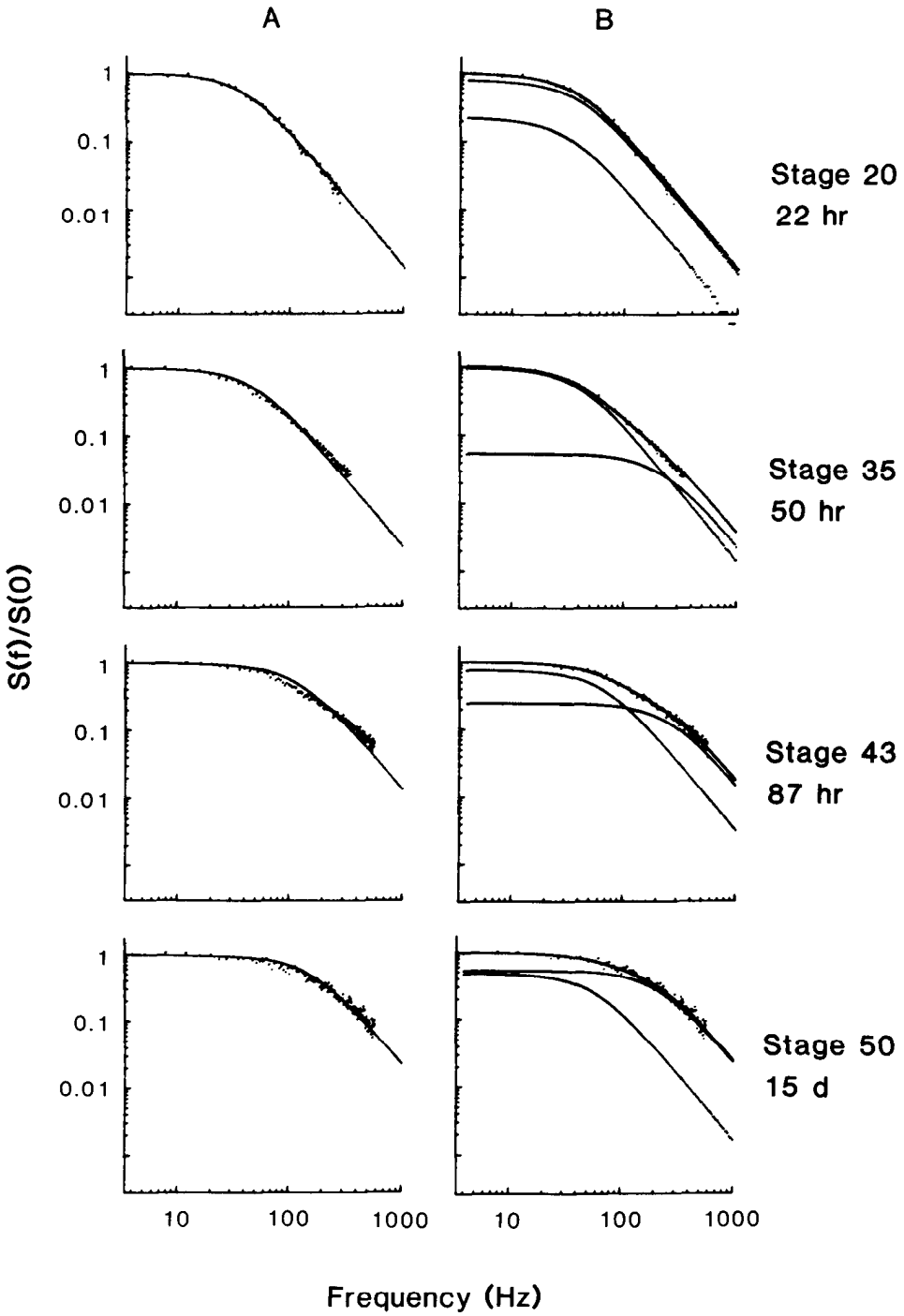


Figure 1. Power density spectra of ACh-induced noise at four developmental stages. The ages correspond to Nieuwkoop and Faber (1967) stages 20, 35, 43, and 50. *A*, Spectra on the *left* have been fitted by single Lorentzian functions. The corner frequencies (hertz) from *top* to *bottom* are: 38, 49, 119, and 152. *B*, The same spectra are fitted on the right by double Lorentzian functions. The separate components are shown as well as their sum. The corner frequencies (hertz) are 32 and 40 (stage 20), 40 and 220 (stage 35), 68 and 260 (stage 43), and 62 and 220 (stage 50).

according to the equation, $\tau = 1/(2\pi f_c)$. The two classes of channels had apparent mean open times of 3.2 and 0.87 msec.

If the receptors present on *Xenopus* myotomal muscle belong to two such classes, then the developmental change in spectral shape should reflect a change in relative frequencies of fast and slow channel openings. In the case of spectra in which both classes of channels contribute to the noise, it is possible to measure the relative frequencies of slow and fast openings, using an equation for the zero frequency asymptote, $S(0)$ (Anderson and Stevens, 1973; Brehm et al., 1982). For two channel types, we have

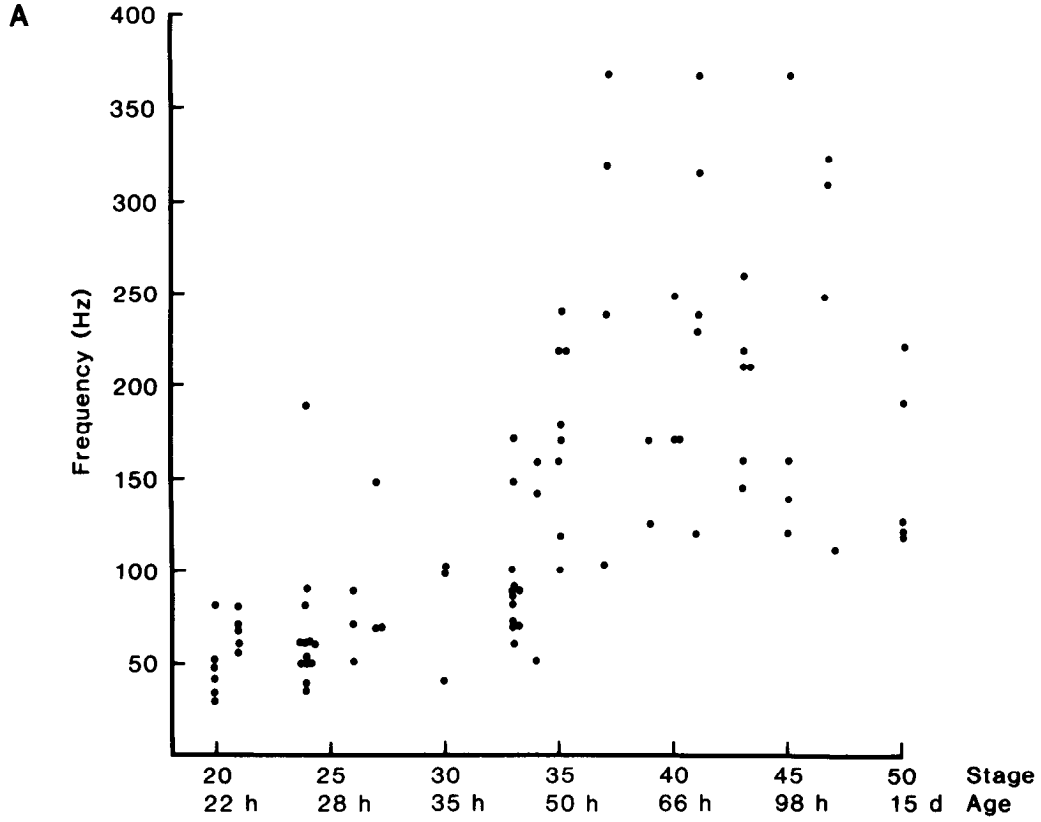
$$S(0)_f \propto N_f(\gamma_f\tau_f)^2$$

$$S(0)_s \propto N_s(\gamma_s\tau_s)^2$$

where the subscripts f and s designate fast and slow variables, N = number of channel openings per second, γ = channel conductance, and τ = apparent mean channel open time. Solving for the fraction, F, of channel openings belonging to the fast class, we have

$$F = N_f/(N_f + N_s) = 1/[1 + S(0)_s\gamma_f^2\tau_f^2/(S(0)_f\gamma_s^2\tau_s^2)]$$

We assumed that the conductance of the faster channel is 1.5 times that of the slower, as found in single-channel recordings of *Xenopus* myotomal muscle *in vivo* (Brehm et al., 1984). The relative frequency of fast channel openings was determined for each spectrum (Fig. 4). When the double Lorentzian corner frequencies were both ≤100 Hz, we assumed that the spectra arose exclusively from slow channel activity, and when the corner frequencies were both >100 Hz, we assumed that the channel activity was exclusively fast. At stages earlier than 30, the fast channel activity was so low in most cases that the spectra appeared to arise only from slow channel activity. There were occasional exceptions to that rule, but it was



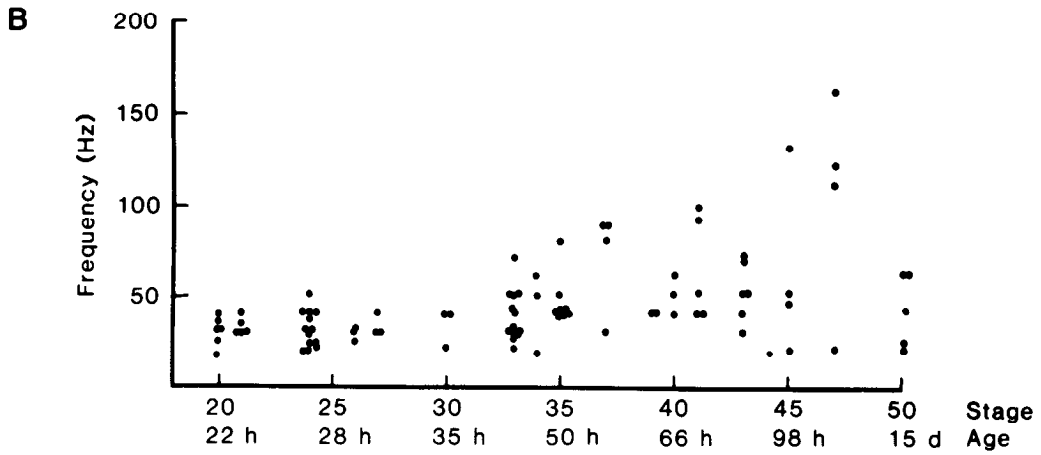


Figure 2. Corner frequencies of double Lorentzian curves versus stage of development. Each *data point* represents a corner frequency estimated from a single spectrum. *A*, High corner frequencies; *B*, low corner frequencies.

not until after stage 33/34 that the fast channel activity was significant in most recordings. There was a wide scatter of values, but a trend was evident of gradually increasing frequency of fast openings with age from stage 33/34 to 50. At stages 45 and older, most recording sites exhibited more than 80% fast channel openings, although occasional sites had relatively little fast channel activity.

We have confined our recordings of ACh noise to nonjunctional regions of membrane because we were concerned that junctional recordings might include noise from nearby nonjunctional receptors. However, we wished to compare the nonjunctional receptor kinetics with those of junctional receptors. An upper limit for the junctional channel open time can be estimated from the exponential decline of synaptic currents, under conditions where the rise and fall of cleft concentration of transmitter are much briefer than the mean channel open time (Anderson and Stevens, 1973; Feltz et al., 1977). Such conditions should exist at the mature myotomal synapse where acetylcholinesterase is abundant (Kullberg et al., 1980). We examined the decay phases of miniature endplate currents (MEPCs), recorded extracellularly, from 52 sites at stages 47 to 50. The decay phases were fitted with single or double exponential curves by least squares.

At 44 recording sites, the decay phases of the MEPCs were fitted well by single exponential curves (Fig. 5*A*). The average time constant was 0.81 ± 0.17 msec, which is comparable to the apparent channel open time of the fast nonjunctional ACh receptors at the same stages. At these locations, there was no detectable deviation from a single exponential throughout the period of decline (beyond 90% of peak amplitude), as judged by eye. The smallest contribution of a slow component which we would have seen is about 20%, based on fitting of theoretical double exponential decays. Because of the smaller conductance of slow channels, a 20% amplitude component corresponds to about 30% slow channel openings. We conclude that at least 70% of the channel openings at these sites were fast. At seven sites, we found MEPCs which

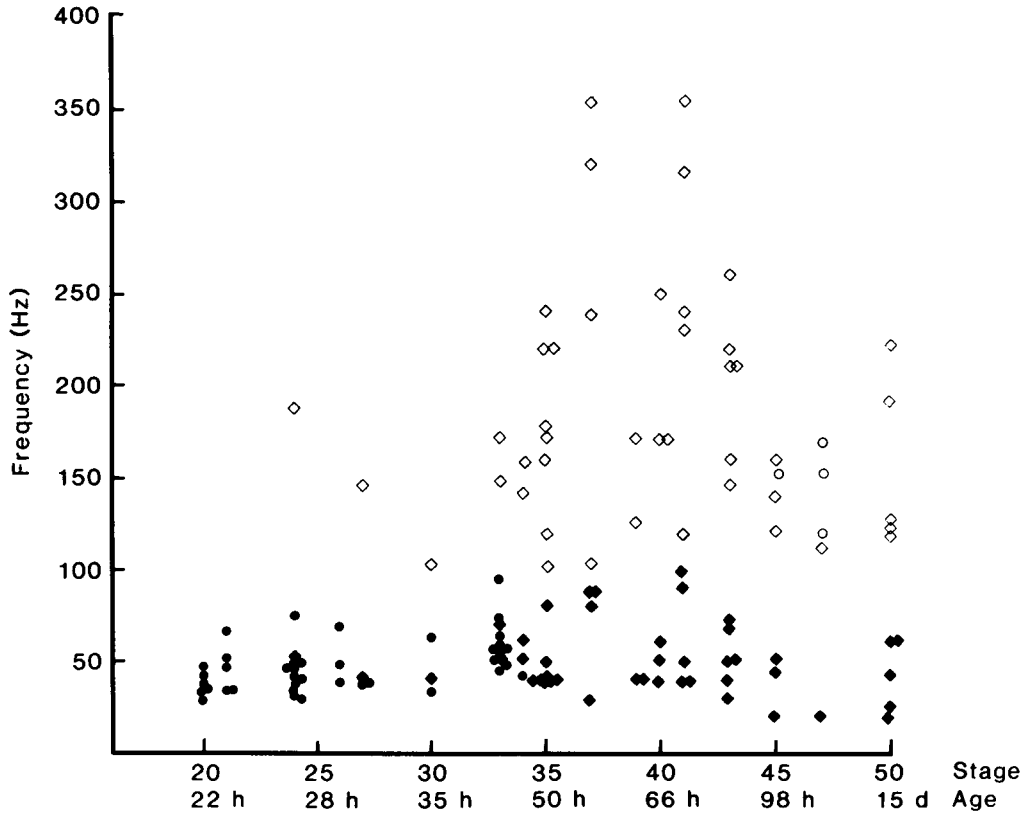


Figure 3. Corner frequencies of spectra from fast and slow channels versus stage of development. *Solid symbols* indicate corner frequencies ≤100 Hz; *open symbols* indicate those >100 Hz. In spectra where the double Lorentzian corner frequencies were both slow (≤100 Hz) or both fast (>100 Hz), it was assumed that the spectra arose from a single class of channels, and the single Lorentzian corner frequencies are displayed (●, ○). In all other cases, the double Lorentzian corner frequencies are displayed (◆, ◇).

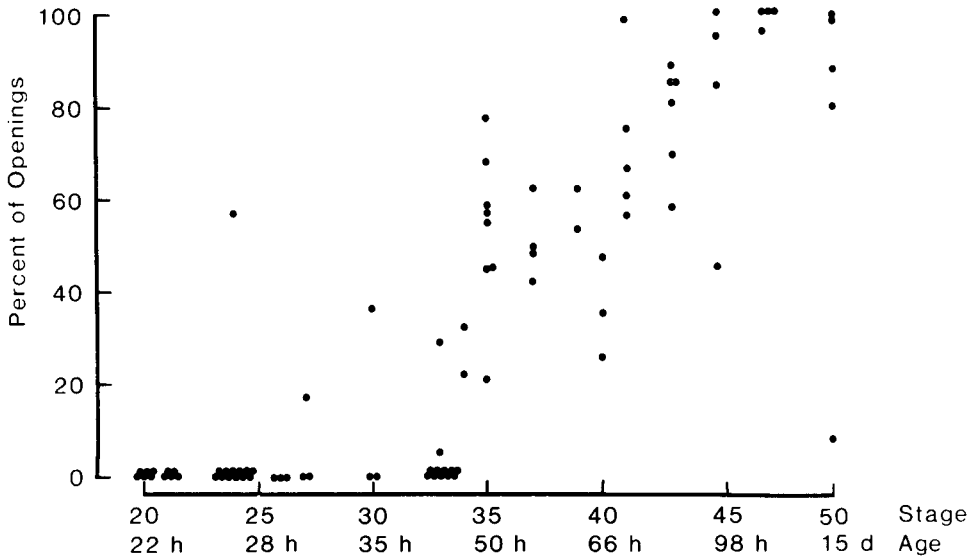


Figure 4. Increase in number of fast channel openings with development. Each data point represents the percentage of fast channel openings contributing to a single spectrum. Fast channel openings were defined as those corresonding to a Lorentzian component with a corner frequency >100 Hz. *Data points* were calculated as described in the text.

deviated noticeably from a single exponential (Fig. 5*B*). They were fitted satisfactorily with double exponential curves having a mean slow component of 2.7 ± 0.6 msec and a mean fast component of 0.78 ± 0.15 msec. On the average, the fast component contributed 44 ± 18% of the total amplitude of the MEPCs. That figure translates into 34% fast channel openings because of the larger conductance of the fast channels. The remaining site had two types of MEPCs, one fast and the other slow (Fig. 5*C*), both fitted well by single exponentials with values of 0.88 and 4.5 msec. It appears that junctional regions had both slow and fast channel types with gating kinetics comparable to those found at nonjunctional regions, and that the slower type of channel was predominant at about 15% of the junctions studied.

Discussion

The spectra of ACh-induced noise at nonjunctional regions of myotomal muscle in *Xenopus laevis* undergo a change in shape during development which is consistent with the idea that a class of embryonic receptors with long open times is largely replaced by, or converted to, a class of mature receptors with fast gating kinetics. This pattern of developmental change is similar to that seen in junctional ACh receptors of developing rat skeletal muscle (Sakmann and Brenner, 1978; Fischbach and Schuetze, 1980; Michler and Sakmann, 1980) and differs from developing chick muscle where no change in open time occurs (Schuetze, 1980).

Receptors first become plentiful on the membrane of *Xenopus* myotomal muscle at stages 19 and 20 (20¾ hr to 21¾ hr after

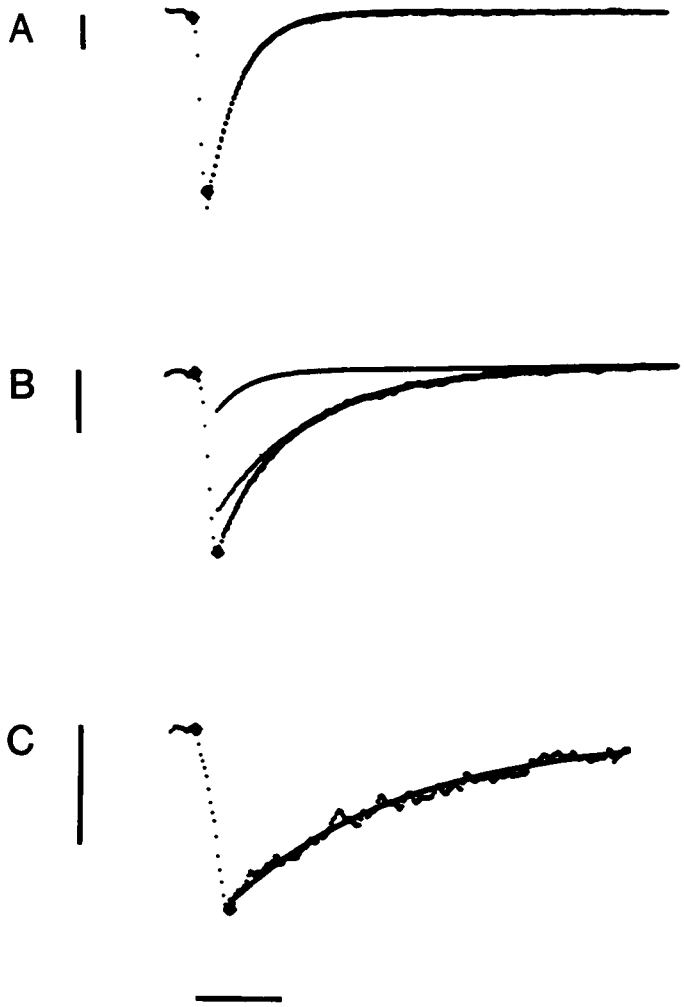


Figure 5. MEPCs recorded at late stage myotomal synapses. Each *trace* is a computer average of 5 to 20 individual MEPCs. The majority of MEPCs were of the type shown in *A*, having a single exponential decay. At a few recording sites, double exponential MEPCs were seen (*B*), and one site had both slow (*C*) and fast (not shown) single exponential MEPCs. Estimated decay constants of the above MEPCs are: *A*, 0.77 msec; *B*, 0.75 and 2.3 msec; and *C*, 4.5 msec. *Vertical bars* = 0.1 mV; *horizontal bar* = 2 msec.

fertilization) according to physiological and autoradiographical studies (Blackshaw and Warner, 1976; Kullberg et al., 1977; Chow and Cohen, 1983). Since our earliest recordings were from stage 20, probably the majority of ACh receptors from which we recorded activity at this stage were present in an active form on the membrane for no more than 1 to 2 hr. Although some of the double Lorentzian curves fitted the spectra better than the single Lorentzian curves during this early period of development, we propose that the ACh receptors at most recording sites were homogeneous with respect to open time. In all but a few spectra, the high and low corner frequencies were relatively close together, and small deviations from single Lorentzian behavior may be explained by experimental error such as imperfect background subtraction or sampling variability of the noise. The double Lorentzian curves, with two additional free parameters, would be expected to fit better on the average than single Lorentzian curves. We do not rule out the presence of fast channels at these early stages but suggest that their frequency of opening, if they were present, was so slow in most cases that they did not contribute substantially to the spectra. Following stage 33/34, there was an appearance of a fast component in the spectra with a corner frequency greater than 100 Hz, and it was no longer possible to fit most of the spectra adequately by single Lorentzian curves. We propose that this higher frequency component represents the activity of a class of channels with faster gating kinetics than those which first appeared on the membrane.

At about the time the high frequency component began to shift upward, the low frequency component also increased (Fig. 2*B*). This shift is probably due to our curve fitting routine, rather than to an actual change in the kinetics of the slow class of channels. That idea was tested by adding a single Lorentzian component with a corner frequency of 200 Hz to six representative spectra from early stages (20 to 24). Before addition of the 200-Hz component, the average low and high corner frequencies were 37 and 68 Hz, and the average best-fitting single Lorentzian curve had a corner frequency of 54 Hz. After addition of the 200-Hz component, the low corner frequency was shifted up to 53 Hz and the high corner frequency to 199 Hz. It appears that when a double Lorentzian curve is fitted to a spectrum which is approximately single, the high and low corner frequencies fall on either side of the best-fitting single Lorentzian corner frequency. With the addition of greater high frequency power, the fast component of the double Lorentzian curve is required to move upward, whereas the slow component begins to approximate the former best-fitting single Lorentzian curve.

Our interpretation of the spectral data is summarized in Figures 3 and 4. We define two classes of channels with apparent mean open times of 3.2 msec and 0.87 msec. For about the first 20 hr following onset of ACh sensitivity, the predominant channel observed on the membrane was the slow form. Shortly before the time of hatching, the faster channel began to contribute significantly to the noise recordings. During the next 2 days, there was an increasing contribution of fast channel activity. At an age when junctional transmission was mature, the predominant nonjunctional channel was the fast form. Even at maturity, however, a measurable fraction (typically less than 20%) of slow channel activity remained.

We are aided in the interpretation of our spectra by the results of single-channel recordings of ACh-activated channels in late stage myotomal muscle (Brehm et al., 1984). In single-channel recordings, two classes of ACh receptors were detected with differing conductances and kinetics. One class had a conductance of 64 pS and a mean open time of 0.70 ± 0.22 msec at −95 mV, whereas the other had a conductance of 44 pS and a mean open time of 1.9 ± 0.9 msec. These open times, particularly that of the slow channel, are briefer than the corresponding estimates of 0.87 and 3.2 msec from ACh noise spectra. The differences may be due to bursting activity of the channels, which can be accounted for to some extent in the analysis of single-channel events. Bursting will lower the corner frequency of the spectrum and lead to an overestimate of the actual channel open time (Colquhoun and Sakmann, 1981; Dionne and Leibowitz, 1982). Another source of error arises from the need to select noise recordings which had large enough amplitudes to yield spectra with good resolution. Since the variance of the ACh noise is proportional to the mean channel open time (Anderson and Stevens, 1973), we are more likely to obtain suitable recordings from slow channel activity than fast, resulting in a bias to overestimate the channel open time. For these reasons, we use the term "apparent open time" to describe our estimate of channel open time.

A major drawback of spectral analysis of ACh noise, as compared to single-channel recording, is that the conductance differences between the slow and fast channel types cannot be used to separate their spectral components. This results in the need to make a somewhat arbitrary division of corner frequencies into slow and fast classes. We have selected 100 Hz as a dividing point, based on the distribution of high corner frequencies. That dividing point may be in error and could contribute to differing estimates of channel open time between spectra and single-channel recording. In spite of these differences, it is a reasonable assumption that the two spectral components found in our noise recordings correspond to the two channel types seen in patch recordings.

The development of ACh receptor gating kinetics in cultured *Xenopus* muscle has been examined in two laboratories by single-channel recording. Both groups used muscle dissociated from

embryos at stage 17 or earlier, which precedes the appearance of ACh sensitivity. Brehm et al. (1983) reported the existence of two classes of channels with open times of 2.2 msec and 0.85 msec and conductances of 42 and 60 pS, respectively (at 20°C and resting potential). In cultures less than 1 day old, more than 90% of channel openings belonged to the slow class; with age, the relative frequency of slow channel openings declined and, in 6-day cultures, only 37% of the openings were slow. They reported no change in apparent mean open time of the two classes of channels with age of the cultures. These results are compatible with our findings, with the exception of differences in the estimates of slow channel open time. Low conductance, slow channels and high conductance, fast channels were also described by Leonard et al. (1983), but they reported a change in kinetics of each class during development. Apparent mean open times were widely separated in the youngest cultures (6.5 msec and 30 msec at 13°C and 50 mV hyperpolarization), whereas in cultures corresponding in age to stage 48 (1 week old), there was less than a 2-fold difference in open times (4.4 msec and 7.2 msec). Differing experimental conditions complicate the comparison of these results with ours; nevertheless, such a convergence of channel open time during development is not suggested by our spectra. In fact, the corner frequencies became more widely separated with age (Fig. 2). However, our spectra do not reveal any information about the association between gating time and channel conductance, and, consequently, we cannot rule out a possible change in the gating time of either conductance class.

In developing rat skeletal muscle, the changes in kinetics of junctional ACh receptors occur simultaneously with the development of postjunctional folding, and it has been suggested that a close relation exists between the two (Brenner and Sakmann, 1983). Our observation of kinetic changes in nonjunctional regions, where folding is not expected to occur, argues against a causal relation between folding and channel gating time. This argument is reinforced by the fact that single-channel recordings from the fast class of ACh receptors can be obtained from mature myotomal muscle over the entire nonjunctional surface (Brehm et al., 1984). It may be that junctional folding is less important in *Xenopus* myotomal muscle than in rat skeletal muscle. The mature myotomal junctional membrane is not deeply folded, as in other vertebrate skeletal muscle, but instead has only shallow ridges of about 0.1 μm in depth (Kullberg , 1977).

Our results suggest that the control of ACh receptor kinetics at late developmental stages (47 to 50) is similar at junctional and nonjunctional regions. At most junctional recording sites, MEPCs decayed in a single exponential fashion with a time constant similar to the apparent fast channel open time at nonjunctional regions. In those cases where MEPCs did not decay as a single exponential, the fast and slow components were similar to the apparent channel open times of the fast and slow receptors at nonjunctional regions. Although the predominant active channel at both junctional and nonjunctional sites was the fast form, there were occasional sites at both locations which were dominated by slow kinetics. The present results give us no reason to believe that there are large differences between the gating times or relative numbers of slow and fast channels at junctional and nonjunctional regions in mature myotomal muscle.

References

Anderson, C. R., and C. F. Stevens (1973) Voltage-clamp analysis of acetylcholine produced end-plate current fluctuations at frog neuromuscular junction. J. Physiol. (Lond.) *235:* 655–691.

Blackshaw, S., and A. Warner (1976) Onset of acetylcholine sensitivity and endplate activity in developing myotome muscle of *Xenopus*. Nature *262:* 217–218.

Brehm, P., R. Kullberg, and F. Moody-Corbett (1984) Properties of nonjunctional acetylcholine receptor channels on innervated muscle. J. Physiol. (Lond.) *350:* 631–648.

Brehm, P., J. H. Steinbach, and Y. Kidokoro (1982) Channel open time of ACh receptors on *Xenopus* muscle cells in dissociated cell culture. Dev. Biol. *91:* 93–103.

Brehm, P., F. Moody-Corbett, and Y. Kidokoro (1983) Developmental alterations in acetylcholine receptor channel properties proceed in the absence of innervation. Soc. Neurosci. Abstr. *9:* 1180.

Brenner, H., and B. Sakmann (1983) Neurotrophic control of channel properties at neuromuscular synapses of rat muscle. J. Physiol. (Lond.) *337:* 159–171.

Chow, I., and M. W. Cohen (1983) Developmental changes in the distribution of acetylcholine receptors in the myotomes of *Xenopus laevis*. J. Physiol (Lond.) *339:* 553–571.

Colquhoun, D., and B. Sakmann (1981) Fluctuations in the microsecond time range of the current through single acetylcholine receptor ion channels. Nature *294:* 464–466.

Dionne, V. E., and M. Leibowitz (1982) Acetylcholine receptor kinetics. A description from single-channel currents at snake neuromuscular junctions. Biophys. J. *39:* 253–261.

Feltz, A., W. A. Large, and A. Trautman (1977) Analysis of atropine action at the frog neuromuscular junction. J. Physiol. (Lond.) *269:* 109–130.

Fischbach, G. D., and S. M. Schuetze (1980) A post-natal decrease in acetylcholine channel open time at rat end-plates. J. Physiol. (Lond.) *303;* 125–137.

Kullberg, R., T. Lentz, and M. Cohen (1977) Development of myotomal neuromuscular junction in *Xenopus laevis*: An electrophysiological and fine-structural study. Dev. Biol. *60:* 101–129.

Kullberg, R. W., F. S. Mikelberg, and M. W. Cohen (1980) Contribution of cholinesterase to developmental decreases in the time course of synaptic potentials at an amphibian neuromuscular junction. Dev. Biol. *75:* 255–267.

Kullberg, R., P. Brehm, and J. H. Steinbach (1981) Nonjunctional acetylcholine receptor channel open time decreases during development of *Xenopus* muscle. Nature *289:* 411–413.

Leonard, R. J., Y. Nakajima, S. Nakajima, and T. Takahashi (1983) Single channel open times of two types of ACh receptors in cultured *Xenopus* myocytes decrease during development. Soc. Neurosci. Abstr. *9:* 1180.

Michler, A., and B. Sakmann (1980) Receptor stability and channel conversion in the subsynaptic membrane of developing mammalian neuromuscular junction. Dev. Biol. *80:* 1–17.

Nieuwkoop, P. D., and J. Faber (1967) *Normal Table of Xenopus laevis*, Ed. 2, Elsevier-North Holland Publishing Co., Amsterdam.

Sakmann, B., and H. R. Brenner (1978) Change in synaptic channel gating during neuromuscular development. Nature *276:* 401–402.

Schuetze, S. M. (1980) The acetylcholine channel open time in chick muscle is not decreased following innervation. J. Physiol. (Lond.) *303:* 111–124.

Schuetze, S. M., E. F. Frank, and G. D. Fischbach (1978) Channel open time and metabolic stability of synaptic and extrasynaptic acetylcholine receptors on cultured chick myotubes. Proc. Natl. Acad. Sci. U. S. A. *75:* 520–523.