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THE ACETYLCHOLINE CHANNEL OPEN TIME IN CHICK MUSCLE IS NOT DECREASED FOLLOWING INNERVATION

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SUMMARY

1. The mean channel open time (τ) of ACh receptors was measured in chick muscles at various stages of development. τ was estimated by analysing ACh induced current fluctuations recorded extracellularly from small (*ca.* 20 μm^2) membrane patches.

2. At random sites on uninnervated, embryonic chick muscle fibres *in vitro*, τ was relatively long – 4 msec at 23 °C.

3. Estimates of τ at synaptic sites on embryonic myotubes innervated *in vitro* were identical to estimates at extrasynaptic sites on the same fibres. Both were comparable to estimates on uninnervated myotubes.

4. Synaptic currents at cultured junctions decayed slowly as simple exponentials. The decay time constants were never shorter than the mean channel open time measured by fluctuation analysis.

5. In anterior latissimus dorsi and intercostal muscle fibres of 4- to 18-week posthatched chicks, fluctuation analysis and synaptic current decays indicate that the channel open time of mature chick endplate receptors is as long as that of embryonic synaptic receptors *in vitro*. Apparently, τ remains prolonged throughout the maturation of chick neuromuscular junctions.

INTRODUCTION

Fluctuation analysis studies have shown that acetylcholine (ACh) receptors in embryonic chick muscle fibres *in vitro* have a mean channel open time of about 4 msec at 23 °C (Sachs & Lecar, 1973; Lass & Fischbach, 1976; Schuetze, Frank & Fischbach, 1978). This value is similar to that reported for extrajunctional ACh receptors in denervated adult muscles in other species (Sakmann, 1975, 1978; Neher & Sakmann, 1976*a*; Dreyer, Walther & Peper, 1976). An earlier report from this laboratory (Schuetze *et al.* 1978) demonstrated that the gating time of embryonic chick receptors is independent of receptor density. On uninnervated myotubes, identical channel open times were measured whether receptors were diffusely distributed at low density (*ca.* 900/μm^2; cf. Sytkowski, Vogel & Nirenberg, 1973) or

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packed within small clusters at a roughly tenfold higher density. Clustered and non-clustered receptors were assayed independently by recording ACh induced current fluctuations from small (*ca.* 20 μm^2) membrane patches with a focal extracellular technique (cf. Neher & Sakmann, 1976*b*).

When embryonic chick myotubes are innervated *in vitro* by spinal cord neurons, new receptor clusters are induced at sites of transmitter release (Frank & Fischbach, 1977, 1979). The channel open time of these newly clustered receptors was also found to be 4 msec at 23 °C, the same as the gating time of uninnervated receptors (Schuetze *et al.* 1978). This was an unexpected result since endplate receptors in mature animals of several other species have much briefer gating times than extrasynaptic receptors (Sakmann, 1975; Neher & Sakmann, 1976*a*; Cull-Candy, Miledi & Trautmann, 1978). It is possible that a decrease in endplate channel open time takes place long after the initial events in synapse formation, and that newly formed cultured synapses simply need more time to express this phenomenon. Alternatively, it may be that the kinetics of chick ACh receptors never change; the mean channel open time may be long even at mature end-plates.

In the present report, these possibilities are explored by comparing the mean channel open time of receptors in embryonic chick myotubes with the open time of receptors in mature endplates in posthatched chicks. The channel open time was estimated both by fluctuation analysis and by measuring the time constant of synaptic current decay (Magleby & Stevens, 1972*a*, *b*; Anderson & Stevens, 1973). Both measures indicate that the gating time of synaptic receptors remains long at least up to 18 weeks after hatching.

METHODS

Cultures

Muscle cultures were prepared according to the procedure of Fischbach (1972). For each plating, the pectoral muscle was removed from an 11 day chick embryo and minced into small fragments with a pair of fine forceps. The minced fragments were incubated for 30 min at 37 °C in a Ca^{2+}- and Mg^{2+}-free saline (Puck's D_1G) without proteolytic enzymes. The tissue was pelleted in a table-top centrifuge for 8 min at 900 rev/min and resuspended in 8 ml. plating medium (see below). Cells were dissociated by repeatedly (*ca.* × 50) passing the fragments through a Pasteur pipette and the larger tissue fragments were removed by filtering the suspension through a double thickness of lens paper. The filtrate was preplated in three 60 mm Petri dishes for 20 min to reduce the number of fibroblasts (Yaffe, 1968). Cells remaining in suspension after this time were plated in 35 mm tissue culture dishes (200,000 cells/dish), each of which contained a 22 mm, collagen-coated coverslip (Gold Seal no. 0). After 24 hr, the medium was replaced with maintenance medium (see below). Thereafter, the medium was replaced every 2 days. When myoblast fusion was nearly complete (2–3 days), remaining fibroblasts were eliminated by adding cytosine arabinoside (10 μM) to the medium for 24 hr.

Two to five days after the muscle was plated, spinal cord explants were added to some of the cultures (Cohen & Fischbach, 1977). Thin (*ca.* 200 μm) transverse sections were cut from the brachial enlargement of 13–14 day embryonic spinal cords and one section was placed in the centre of each muscle coverslip. Nerve processes grew out from the explants and innervated nearby myotubes within 24 hr.

Media. Plating medium: Eagle's Minimum Essential Medium (made up in Earle's Balanced Salt Solution) supplemented with heat-inactivated horse serum (10% by volume), chick embryo extract (5% by volume), glutamine (2 mM), penicillin (50 u./ml.), and streptomycin (50 μg/ml.). Maintenance medium: as above, except that the level of chick embryo extract was reduced to 2% (by volume).

Muscles from post-hatched chicks

Male, white Leghorn chickens were obtained from Spafas, Inc. Each bird was decapitated, and either the entire anterior latissimus dorsi (a.l.d.) muscle or a strip of intercostal muscle was removed as quickly as possible (*ca.* 10 min). During dissection, the muscles were kept moist with oxygenated Eagle's medium. A.l.d. muscles were studied in two 4 week birds, in three 5 week birds, and in one bird each at 6, 9 and 18 weeks. Intercostal muscles were studied in one 4 week and one 10 week chicken.

The a.l.d. is a multiply innervated slow muscle with *en grappe* end-plates distributed at about 1 mm intervals along each fibre (Ginsborg & Mackay, 1961). This multiple innervation facilitated localization of endplates with extracellular recording. The muscle was removed according to the procedure of Ginsborg (1960) and pinned out, deep side down, in a plastic Petri dish containing a layer of transparent plastic resin (Sylgard 184, Dow Corning). To improve the visualization of individual muscle fibres, the muscle was thinned under a dissecting microscope by peeling off several layers of fibres from the exposed dorsal surface. The muscle was then turned over and pinned dorsal side down to expose the undamaged deep surface.

Two types of end-plates have been described in chick intercostal muscle (Atsumi, 1971). Some fibres receive a single large *en plaque* end-plate while others are innervated at two to thirteen sites by smaller *en grappe* nerve endings. In either case, the short length of each muscle fibre made it relatively easy to record synaptic currents with an extracellular electrode. A strip of intercostal muscle was obtained by cutting out the lateral third of several ribs near the bottom of the cage. A layer of muscle fibres was peeled away from one surface of the ribs, and then the preparation was turned over and pinned out at approximately the normal resting length of the fibres.

Dissociated adult rat muscle fibres

Experiments designed to test the spatial resolution of extracellular current recordings (Pl. 1) were performed on dissociated adult rat muscle fibres. The procedure of Bekoff & Betz (1977*a*) was used to enzymatically dissociate individual, intact muscle fibres from the flexor digitorum brevis muscle of a 50–75 gm Sprague–Dawley female rat. The isolated muscle fibres were plated on collagen coated glass coverslips in maintenance medium (see above) and were studied within 2 days.

Electrophysiology

Coverslips with muscle or spinal cord plus muscle cultures were placed in a glass-bottomed (no. 1 Gold Seal glass coverslip) Plexiglass chamber mounted on the stage of an inverted microscope (Leitz Diavert) equipped with phase contrast and interference contrast optics. Petri dishes containing a.l.d. or intercostal muscles were studied with bright-field optics on an upright microscope (Leitz Ortholux). The entire stage was heated with an infrared lamp to maintain a temperature between 21 and 37 °C. The bath temperature near the preparation was continuously monitored.

Recording solutions. With the exception noted below, all recordings were made in Eagle's Minimum Essential Medium (without serum or embryo extract) equilibrated with 95% O_2/5% CO_2. The bath was replaced at 15 min intervals.

In one series of experiments included in this paper, the bath was an oxygenated mixture of two parts Earle's Balanced Salt Solution and one part complete plating medium (Eagle's Medium plus serum and embryo extract). In general, similar results were obtained in both solutions. However, the mean channel lifetime of AChR's on cultured myotubes was somewhat shorter (by 28% at 30 °C) in the more complex solution than in Minimum Essential Medium alone. The reason for this difference was not pursued.

Fluctuation analysis. Focal extracellular recordings of ACh-induced current fluctuations were made with relatively blunt micropipettes filled with 40 μM-ACh in Earle's Balanced Salt Solution (cf. Neher & Sakmann, 1976*b*). The electrodes were drawn on an Industrial Sciences, horizontal puller with the filament current set very low in order to make the taper of the electrode as rapid as possible. The tip of each pipette was visualized with a compound microscope and broken to an internal diameter of 5–7 μm. Those with jagged or uneven tips were rejected. The tip of each acceptable electrode was reduced to an internal diameter of 3–5 μm by fire-polishing with an electrically heated nichrome wire.

Both DC-coupled and bandpass-filtered (Krohnhite, 0·5–1100 Hz) extracellular voltage signals were displayed on a Gould chart recorder and on a Tektronix 565 or D11 oscilloscope. The filtered signal was also fed to the analog input of a PDP8/E computer for on-line analysis. The signal was sampled at 2·0 kHz and successive 0·512 sec long segments (1024 points) were stored in core memory. Before analysis, each digitized segment was displayed on a monitor oscilloscope, and those with obvious artifacts or with superimposed synaptic potentials were discarded. Acceptable segments were smoothed with a cosine taper (Bendat & Piersol, 1971) and the power spectrum of each was calculated with a fast Fourier transform routine. The amplitudes of adjacent frequencies were averaged to reduce the number of estimates within each spectrum by a factor of 2. Smoothed spectra of seven segments were averaged and a control spectrum (obtained with the recording pipette positioned away from the cell) was subtracted from the mean. Control spectra were essentially flat between 2 and 1000 Hz, with an occasional peak at 60 Hz. Thus, 7168 samples were used to compute 256 spectral estimates linearly distributed between 2 and 1000 Hz.

The half-power frequency ($f_{\frac{1}{2}}$) was calculated by fitting a Lorentzian curve (eqn. (1) in Results) to the net ACh spectrum with a non-linear least-squares technique. Spectral estimates centred at 2 and 60 Hz were excluded, as were all of the higher frequency estimates which had amplitudes less than twice that of estimates in the control spectrum. Generally 60–200 estimates were retained in calculating the half-power frequency; the exact number depended upon the amplitude of the recorded signal and the half-power frequency of the spectrum. To minimize the variance in estimating $f_{\frac{1}{2}}$, each spectral value was weighted by the inverse of its expected variance (Armitage, 1971), taking into account contributions to the variance made by the recording system itself. When this analysis was applied to electronically simulated data, the half-power frequency was determined with an accuracy of better than 1%.

Decay constants of synaptic currents. Focal extracellular recordings of spontaneous synaptic potentials were made with electrodes similar to those used for fluctuation analysis, except that they contained no ACh. Since the time course of extracellular synaptic potentials is identical to the local transmembrane current, the phrase 'synaptic current' will be used to designate extracellularly recorded synaptic potentials.

Recordings were filtered (Krohnhite, 0·5–2000 Hz) and sampled on-line at a rate of 20 kHz by the PDP8/E computer. Digitized records were stored in core memory. Only extracellular potentials with amplitudes $> 200\ \mu$V (about 5 times the peak-to-peak base line noise) were collected; most amplitudes were $> 500\ \mu$V. The samples were distributed such that, for each potential, they included about 2 msec of the base line preceding the rising phase and about 10 msec following the peak.

The decay constant of each current was estimated by fitting a simple exponential to the falling phase using least-squares regression. Only the middle 60% of the falling phase was used for the regression in each case. The upper 20% was omitted due to the curvature near the peak, and the final 20% was excluded due to the poor signal-to-noise ratio. Each synaptic current was displayed on a monitor oscilloscope with the least-squares curve superimposed. If the trace contained (1) a clear artifact or excessive noise or (2) a second synaptic current superimposed on the falling phase of the first, the record was discarded. Correlation coefficients for acceptable fits were always $> 0{\cdot}90$ and usually $> 0{\cdot}97$.

RESULTS

ACh-induced current fluctuations

When an extracellular electrode filled with 40 μM-ACh was lowered onto the surface of a cultured chick myotube, there was a negative-going potential shift of several hundred μV accompanied by a marked increase in potential fluctuations (Fig. 1). Both the steady potential shift and the increased fluctuations reflect the action of ACh: no response was observed when the electrode was pressed against membranes that contained no ACh receptors (fibroblasts) or very few receptors (extrasynaptic sites on innervated adult muscle fibres). Furthermore, no response

was seen when ACh was omitted from the pipette or when the bath contained curare (0·1 mM).

It is likely that only those receptors immediately beneath the ACh pipette tip contribute to the recorded response. The high spatial resolution of the electrode is demonstrated in Pl. 1. In this experiment, the extracellular micropipette was positioned at several sites within and near the endplate region of an isolated adult rat muscle fibre maintained *in vitro*. This cell was examined after only 1 day in culture, that is, before the appearance of significant extrajunctional sensitivity

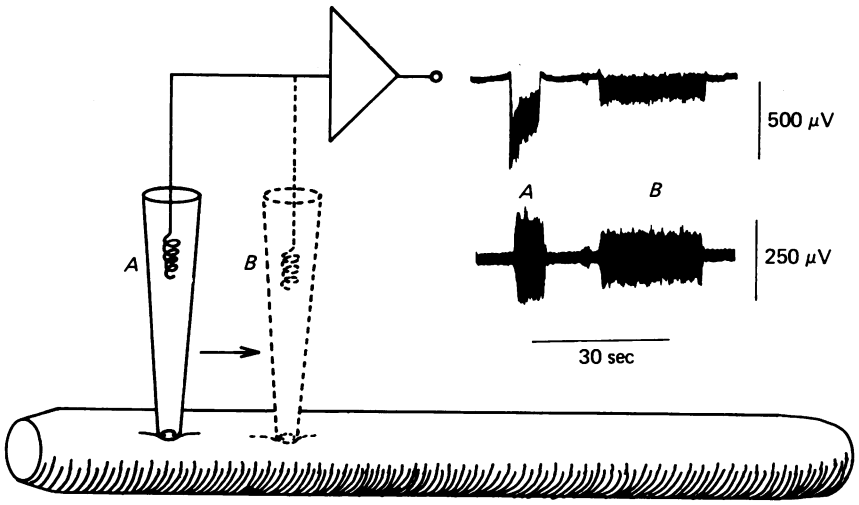

Fig. 1. Extracellular recordings of ACh-induced current fluctuations at two membrane patches (*A* and *B*) on an embryonic chick myotube in culture. The electrode was filled with 40 μM-ACh. The letters between the traces correspond to the positions of the recording electrode. Upper trace, DC-coupled; lower, bandpass-filtered (0·5–1100 Hz).

(Bekoff & Betz, 1977*a*, *b*). A large response was observed when the electrode was placed at the vacated end-plate, but when the electrode was repositioned just a few micrometers away from this site, the signal was lost completely.

Channel open time at sites on uninnervated myotubes

Several studies have shown that the temporal characteristics of ACh induced current fluctuations are determined by the mean channel open time of activated ACh receptors (Katz & Miledi, 1972; Anderson & Stevens, 1973). The power spectrum ($S(f)$) of the fluctuations is a simple Lorentzian curve, defined by

$$S(f) = \frac{S(O)}{1+(f/f_{\frac{1}{2}})^2} \tag{1}$$

where $S(O)$ is the maximum value of $S(f)$, f is frequency, and $f_{\frac{1}{2}}$ is the frequency at which $S(f)$ is half-maximum. This is consistent with a simple model of channel kinetics in which the fluctuations are assumed to reflect random variations in the number of open channels. According to this model, if one assumes that receptors

operate independently and that channel closing is the rate-limiting step in drug-receptor kinetics, then the mean lifetime of an open channel, τ, is given by

$$\tau = 1/(2\pi f_{\frac{1}{2}}). \quad (2)$$

Extracellularly recorded, ACh induced potential fluctuations are directly proportional to the underlying transmembrane current fluctuations and hence yield valid estimates of τ. A typical recording from a chick myotube is shown at a high sweep speed in the upper left of Fig. 2; the corresponding power spectrum is shown

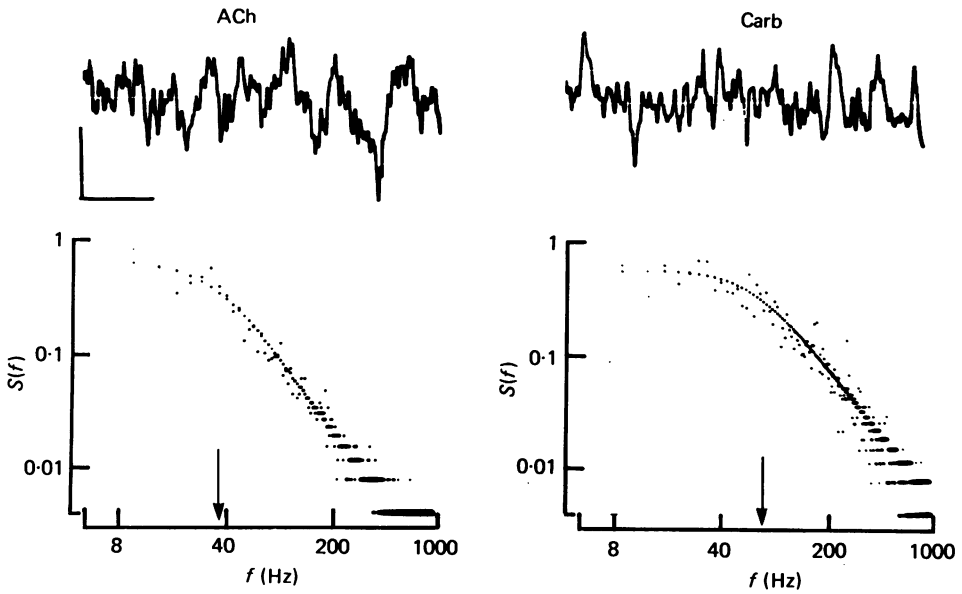

Fig. 2. ACh and carbachol (Carb) induced current fluctuations recorded from the same chick myotube. The corresponding power spectrum is shown below each trace with $S(f)$ in relative units. Superimposed on each experimental spectrum in this and all subsequent figures is a least squares Lorentzian curve (shown as a sequence of dots). The arrows indicate half-power frequencies and correspond to mean channel open times of 4·9 msec (ACh) and 2·2 msec (carbachol). Calibration bars: 100 μV, 20 msec. Temperature = 22 °C.

below. In this case, as in all others, the spectrum was well described by eqn. (1). The half-power frequency ($f_{\frac{1}{2}}$) was 33 Hz, which indicates a mean channel open time (τ) of 4·9 msec.

In frog muscle, the half-power frequency of carbachol induced current fluctuations is shifted to the right relative to the $f_{\frac{1}{2}}$ of ACh induced fluctuations: τ is shorter when the partial agonist carbachol, rather than ACh, activates the receptors (Katz & Miledi, 1973*a*). A similar result was found in chick myotubes (Fig. 2, right).

The estimated mean channel open time was temperature dependent. When recordings were made from random myotube sites with an ACh-filled pipette, τ decreased with temperature from 4·6 ± 0·4 msec (mean ± S.E.) at 21 °C to 0·85 ± 0·04 msec at 37 °C, with a Q_{10} of 3·1 (Fig. 3). The temperature dependence of τ was even greater when carbachol was used as the agonist (Q_{10} = 4·4). Thus, τ_{ACh}/τ_{carb} increased from 1·5 at 21 °C to 2·5 at 33 °C.

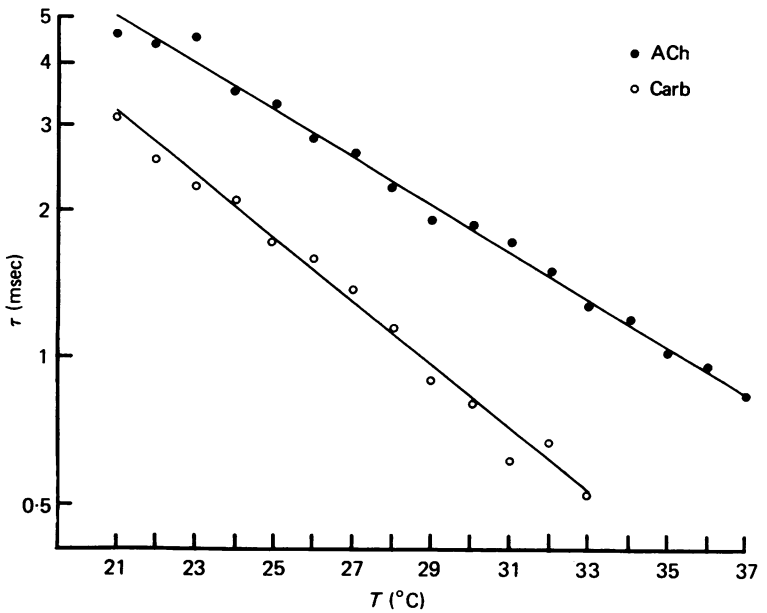

Fig. 3. Temperature dependence of channel open time on uninnervated myotubes. Each point represents the mean of two to fourteen determinations (usually six) at different randomly selected sites; the normalized standard error of each is $\pm 10\%$ or less. The recording electrode contained 40 μM-ACh (filled circles) or 400 μM-carbachol (open circles). The straight lines were fit by least-squares regression.

TABLE 1. Channel open time in cultured chick myotubes estimated with different concentrations of ACh in the extracellular pipette. All measurements were made in the same culture. Temperature = 22 °C

ACh concn.	No. of cells	Mean τ (msec)	Standard deviation (msec)
20 μM	8	4·4	0·5
40 μM	4	4·4	0·5
80 μM	17	3·9	0·8

Contacting a myotube with a 3 μm diameter pipette filled with 40 μM-ACh elicited a negligible depolarization. In twenty cells, the membrane potential was monitored with an intracellular electrode during extracellular recordings of ACh fluctuations. The maximum intracellular potential change recorded within 50 μm of the extracellular ACh pipette was always less than 1 mV, even at the most sensitive sites. Thus, all estimates of τ reported here refer to the normal myotube resting potential (-67 ± 1 mV; mean $\pm$ S.E.).

Estimates of τ did not depend on the concentration of ACh within the pipette. Identical estimates were obtained with pipettes filled with 20 or 40 μM-ACh, though the lower concentration induced somewhat smaller currents (Table 1). When higher levels of ACh (80 μM) were used, estimates of τ at most sites were unchanged. At four out of seventeen sites, however, τ was shorter with 80 μM-ACh than with 40 μM-ACh. In these cases, leakage of ACh out of the pipette was probably sufficient

to depolarize the membrane; a slow, local contraction invariably developed as the electrode approached the cell surface. Since channel open time decreases with depolarization (Anderson & Stevens, 1973), the four low estimates of τ probably reflect a decline in membrane potential rather than a direct concentration-dependent effect of ACh.

Fluctuation analysis gives an accurate estimate of τ only if the dose of ACh is well below that level which saturates the receptors (Anderson & Stevens, 1973; Neher & Sakmann, 1976*a*). A simple calculation demonstrates that this constraint was satisfied in the present experiments. A 40 μM-ACh-filled pipette depolarized the membrane by only 1 mV at most. Assuming a myotube input resistance of $10^7\ \Omega$ (Fischbach, Nameroff & Nelson, 1971), an ACh driving force of 50 mV, and a single channel conductance of 30 pmhos (Lass & Fischbach, 1976), it follows that a 1 mV ACh-induced depolarization represents the summed effect of no more than 100 open channels. Since the extracellular pipette covers about 20 μm^2 of surface membrane containing a total of roughly 18,000 ACh receptors (cf. Sytkowski *et al.* 1973), it follows that less than 1% of the available channels must be open at any given instant.

τ *at sites of transmitter release*

When spinal cord explants were added to established muscle cultures, most of the myotubes near the explant were innervated by outgrowing neurites within 1–3 days. The precise location of newly formed synapses was determined by focal extracellular recording of synaptic currents. The ACh filled electrode was repositioned at each synapse until the amplitude of synaptic currents was maximal. The optimal region about each synapse was small: a 3 μm lateral displacement resulted in a twofold decline in the recorded size of synaptic currents, even when the pipette was moved along the course of the overlaying neurite.

ACh induced current fluctuations were recorded from fourteen different synaptic sites. As previously reported (Schuetze *et al.* 1978) the mean synaptic channel open time estimated from ACh spectra was long (3·0–4·0 msec) at 24 °C. The ratio of τ at synapses to τ at nearby extrasynaptic sites on the same myotubes was $1{\cdot}1 \pm 0{\cdot}1$ ($n = 14$) over a temperature range of 23–30 °C.

Analysis of synaptic current decay rates also indicated that the gating time of channels at chick synapses *in vitro* is prolonged. Several records from one synapse are shown in Fig. 4. As the bath temperature was increased from 24 to 34 °C, the exponential decay time constant (τ_s) decreased from 3·3 to 1·3 msec. This temperature dependence ($Q_{10} = 2{\cdot}5$) is about the same as that found for channel open time by fluctuation analysis. At most of 100 synapses studied, the mean τ_s was virtually identical to the mean channel open time estimated at the same site by fluctuation analysis. At some synapses, however, τ_s was significantly *longer* than the channel open time, but in these cases (in contrast to the others) histochemical staining failed to reveal localized deposits of acetylcholinesterase (Rubin, Schuetze & Fischbach, 1979). Thus, the slow synaptic currents at such synapses were probably due to slow removal of ACh, and not to a subpopulation of ACh receptors with unusually long channel open times.

τ in post-hatched chicks

Junctions in anterior latissimus dorsi (a.l.d.) and intercostal muscles removed from post-hatched chicks were located by extracellular recording, as described above. In the multiply innervated a.l.d. muscle (Ginsborg, 1960), junctions could be found along the entire muscle length. In the intercostal muscle, which contains many singly innervated fibres (Atsumi, 1971), an endplate-rich zone was located

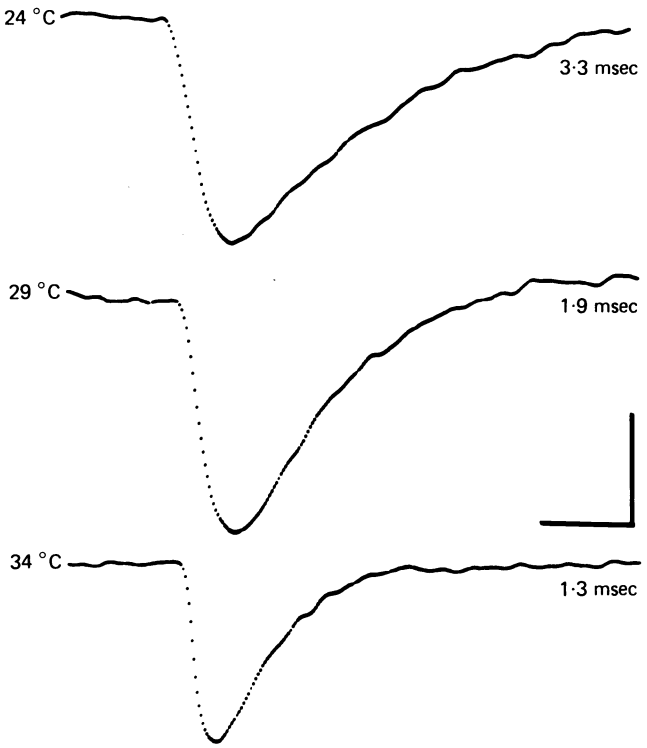

Fig. 4. Temperature dependence of synaptic currents at a nerve-muscle synapse that formed in culture. The number to the right of each trace is the decay time constant (τ_s). Calibration bars: 500 μV, 2 msec.

by intracellular recording of m.e.p.p.s with fast rising phases. Bursts of synaptic currents were recorded from some fibres. They presumably reflected damage inflicted by the extracellular electrode, and such fibers were not studied further.

In 4 week chicks, the mean ACh channel open times at a.l.d. and intercostal junctions were $5{\cdot}2 \pm 0{\cdot}3$ msec (mean $\pm$ S.E.) and $5{\cdot}7 \pm 0{\cdot}3$ msec, respectively, at 21 °C. These values are comparable to τ's found at newly formed nerve-muscle synapses in culture assayed at the same temperature. Experiments on older chicks revealed that τ remained unchanged up to 10 weeks post-hatching in intercostal fibres and up to 18 weeks post-hatching in a.l.d. fibres (Fig. 5). The temperature dependence of τ in the two muscles was the same as that seen in cultured embryonic muscle.

In both a.l.d. and intercostal muscles, ACh induced current fluctuations frequently

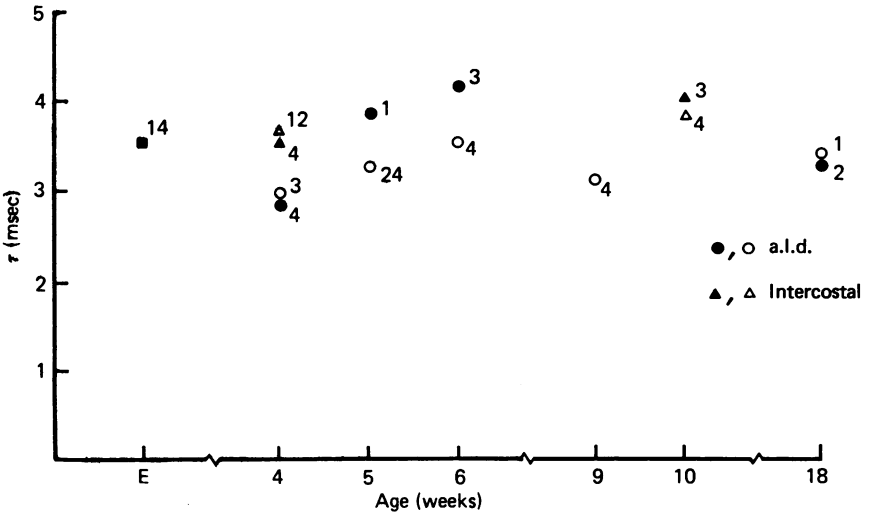

Fig. 5. Mean channel open time of ACh receptors in a.l.d. (circles) and intercostal muscle fibres (triangles) as a function of age. Filled symbols: sites with spontaneous synaptic currents. Open symbols: sites without spontaneous synaptic currents (see text). Numbers indicate the number of fibres examined. For comparison, the average τ at synaptic sites on cultured embryonic myotubes is given above the E on the abscissa (filled square). All values normalized to 25 °C.

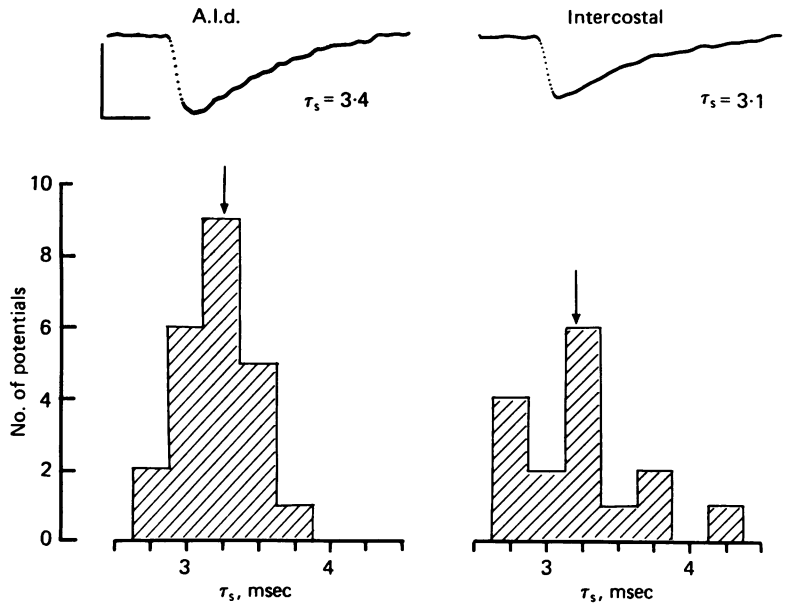

Fig. 6. Synaptic currents at a.l.d. and intercostal end-plates. Below each trace is the histogram of synaptic current decay constants at the same site. Arrows indicate the mean decay constants (a.l.d., $\bar{\tau}_s$ = 3·3 msec at 25 °C; intercostal, $\bar{\tau}_s$ = 3·2 msec at 27 °C). Calibration bars: 500 μV, 2 msec.

were recorded at sites that did *not* exhibit spontaneous synaptic currents. In virtually all such cases, however, synaptic currents were recorded at one or more nearby regions. It is not known whether the inactive recording sites were in perijunctional regions or at junctional sites that released transmitter infrequently. In either event, the mean channel open time measured at inactive sites (Fig. 5, open symbols) was not significantly different from that determined at sites with spontaneous synaptic currents (filled symbols).

Synaptic current decay constants were determined at six a.l.d. and three intercostal junctions in 4 week-old chicks. Examples are shown in Fig. 6, along with histograms of the decay constants. In each case, the mean decay constant of synaptic currents was similar to the mean channel open time estimated at the same temperature by fluctuation analysis.

DISCUSSION

Chick muscles are innervated *in ovo* about two weeks before hatching (Landmesser & Morris, 1975). Thus the prolonged gating time of chick synaptic receptors persists for at least 20 weeks after synapse formation. Identical estimates of τ were found in all preparations tested: uninnervated cultured embryonic myotubes; innervated, cultured myotubes at both synaptic and extrasynaptic sites; and at end-plates in both a.l.d. and intercostal muscles of post-hatched chicks.

In light of results found in other species, the relatively long channel open time measured at junctions of post-hatched chicks is surprising. At junctions in adult frogs (Katz & Miledi, 1972; Anderson & Stevens, 1973), rats (Sakmann, 1975, 1978; Colquhoun, Large & Rang, 1977; Fischbach & Schuetze, 1980), and humans (Cull-Candy *et al.* 1978), the mean channel open time at room temperature is about 1 msec, several-fold shorter than τ in adult chicks. The gating time of chick junctional channels (about 4 msec) is similar to that of *extra*junctional channels in denervated frog (Neher & Sakmann, 1976*a*; Dreyer *et al.* 1976) and rat (Sakmann, 1975, 1978; Fischbach & Schuetze, 1980) muscles.

One might argue that this difference in synaptic channel kinetics is not a species difference at all, but rather a difference between singly innervated (fast twitch) and multiply innervated (slow twitch or tonic) muscles. Chick a.l.d. fibres are all multiply innervated (Ginsborg & Mackay, 1961). Intercostal muscles, however, contain many singly innervated fibres (Atsumi, 1971). One of the intercostal muscles used here (10 weeks post-hatching) was fixed and stained for AChE by the method of Karnovsky & Roots (1964). The majority of the fibres appeared to have only one accumulation of reaction product, and the over-all staining pattern was markedly different from that of a multiply innervated a.l.d. muscle examined under similar conditions. It is unlikely, therefore, that all intercostal recordings were from multiply innervated fibres. In addition, τ estimated at one posterior latissimus dorsi (p.l.d.) end-plate 5 weeks post-hatching was similar to τ in other chick muscles. The p.l.d. contains fast twitch, singly innervated fibres almost exclusively (Ginsborg & Mackay, 1961).

It is unlikely that chick junctions contain a population of fast channels that somehow were missed in these experiments. Even though the ACh-filled pipette was positioned at sites which maximized the signal strength (presumably at synaptic

ACh receptor clusters), all estimates of τ were uniformly prolonged. Complex power spectra, which might indicate a subpopulation of fast channels (cf. Fischbach & Schuetze, 1980), were never observed in any of the sixty-nine a.l.d. and intercostal fibres studied. More significantly, the mean decay time constants of synaptic currents were in good quantitative agreement with the results of fluctuation analysis. Since acetylcholinesterase is present at both a.l.d. (Ginsborg & Mackay, 1961) and intercostal (Atsumi, 1971) junctions, the decay times of synaptic currents should accurately reflect the open time of synaptic channels (cf. Katz & Miledi, 1973*b*; Rubin *et al.* 1979).

In short, the chick is different from other species in terms of the mean channel open time of mature endplate receptors. This exception is important in that it shows that τ is not necessarily linked to several other variables such as receptor density, the fact of innervation, or receptor degradation rate. Identical channel open times were measured both in cultured embryonic myotubes and in mature chick end-plates. In the former case the receptors are present at low density, uninnervated, and degraded rapidly ($t_{\frac{1}{2}} \simeq 1$ day; Devreotes & Fambrough, 1975) whereas in the latter case the receptors are densly packed, innervated for many weeks, and degraded slowly ($t_{\frac{1}{2}} > 5$ days; Burden, 1977). In the following paper it is shown that the same conclusions apply to receptors in the rat, a species in which a developmental decrease in τ does occur (Fischbach & Schuetze, 1980).

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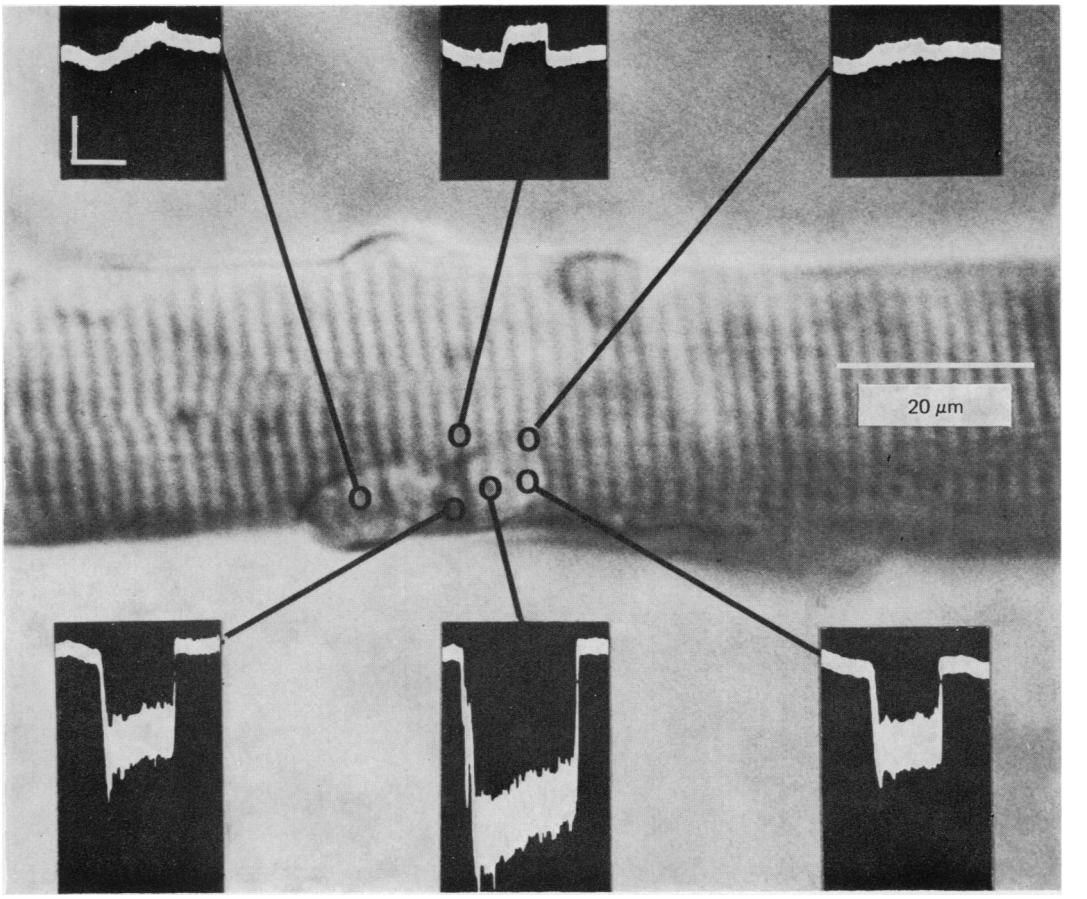
Explanation of plate

Spatial resolution of extracellular recordings. ACh-induced current fluctuations were recorded (DC-coupling) from six membrane patches within and near the end-plate region of an adult rat muscle fibre that was maintained *in vitro* for 24 hr. The nerve terminals were stripped during the dissociation. The circles indicate the position of the electrode tip during each recording. Calibration bars: 200 μV, 5 sec.



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(*Facing p.* 124)