# Spontaneous junctional currents in *Drosophila* muscle fibres: Effects of temperature, membrane potential and ethanol

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Summary. Fast, slow and fast multiquantal spontaneous junctional currents were recorded from glutamate sensitive muscle fibres of *Drosophila* larvae. Decrease of temperature and hyperpolarization prolonged the time course of fast currents. Ethanol (0.4 M) markedly shortened their duration, whereas several other drugs known to modify the time course of currents at cholinergic synapses were ineffective at this neuromuscular junction.

It has recently been found that the duration of miniature excitatory junctional currents (m.e.j.c.s) in locust muscles decreases at a higher temperature and with hyperpolarization[2]. In the present study, the spontaneous excitatory junctional currents (e.j.c.s) of larval muscles of another insect, the fly *Drosophila melanogaster,* were recorded, and their dependence on changes of membrane potential (MP) and temperature was studied. Several drugs and ethanol which are known to modify the amplitude and time course of e.j.c.s at cholinergic neuromuscular synapses (for review see Magazanik[3]) were also tested on this junction, at which L-glutamate was found to act as agonist[4,5].

*Material and methods.* The experiments were performed on the muscle bag of third instar larvae and early pupae stage of the Canton-S strain of flies[4,5]. As a perfusing medium, we used the solution A (see Jan and Jan[4], p. 191). The standard 2-microelectrode technique and a voltage clamp circuit[6] were employed. High frequency noise in the voltage clamp was filtered by a 3-5 kHz low pass Schlumberger filter FAB-48 (96 db/decade roll off) to reduce the noise level to 0.07-0.15 nA. The muscle fibres of *Drosophila* larvae were found convenient for clamp experiments: the fibres are only 0.5 mm long, 80 µm in diameter[4] and have a specific membrane resistance of $4.3 \times 10^3\ \Omega\ cm^{-2}$. Recording and clamping glass microelectrodes were inserted within 20 µm of each other in the middle section of the ventral lateral or ventral segmental muscle fibres.

*Results and discussion.* At 22 °C the mean value of resting MP was found to be 52.0±0.5 mV (number of cells, n=51), which is close to the value previously reported[4]. Figure 1, A, shows miniature e.j.c.s of different amplitude and time course. The 'fast' m.e.j.c.s, with a mean amplitude of 0.41±0.09 (mean±SE) nA, rise time ($t^{rise}$)=1.60±0.03 msec and half-decay time, ($t^{dec}_{1/2}$, estimated as time during which the amplitude of the current decreases to half of the maximum) 3.11±0.13 msec (n=25, MP=50 mV) were the most frequently observed. Besides these, 'slow' miniature e.j.c.s were sometimes present in about one-third of the fibres (similarly to frog slow muscle fibres[7]) with $t^{rise}$ 4-10 msec and $t^{dec}_{1/2}$=7-20 msec (figure 1, A). The mean amplitude and frequency of the slow m.e.j.c.s varied widely between different cells, but they were very often smaller than the fast ones and their frequency was substantially lower (figure 1, A). They were also observed together with other types of e.j.c.s (more complex in shape) in the abdominal segmental muscle fibres of the adult fly (figure 1, C). Because of their great diversity, the slow currents were not examined further. Besides these 2 groups, 'giant', apparently multiquantal e.j.c.s of regular shape and small variability of amplitude (figure 1, B) were observed in 5 fibres in early pupae preparations. They often started to fire in regular trains, with a frequency of about 10 Hz, and sometimes disappeared after a few min. The rise time (1.93±0.03 msec at MP=−50 mV) was found to be closer to $t^{rise}$ of the fast m.e.j.c.s than to the slow ones, and a comparison of the mean amplitudes of both small (fast) and giant currents was therefore used[8] for calculation of their quantum content m (m=26.1±1.3, n=5). When the coefficient of amplitude variation[8] was used to calculate m, a similar value (22.5±1.8) was obtained. On the other hand, the $t^{dec}_{1/2}$ (5.22±0.1) of the giant e.j.c.s was greater by about 60% than that of the fast ones (figure 1, F, G). Because the $\tau$-values for the exponential part of the decay phase were

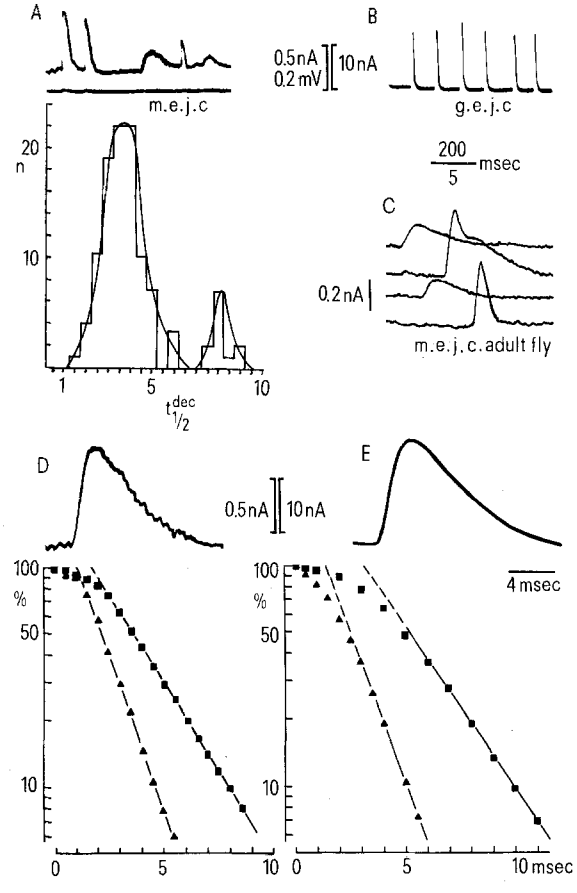


Fig. 1. Spontaneous e.j.c.s of the fly muscle fibres. *A* Fast and slow miniature e.j.c. (m.e.j.c.) on 1 larval fibre and histograms of the distribution of their half decay time ($t^{dec}_{1/2}$, n-number of m.e.j.c.s). Lower trace of the record (scale in mV) indicates the effectiveness of the clamp. *B* An example of spontaneous giant currents (g.e.j.c.) which were found in several fibres. *C* Miniature e.j.c.s of different shape in abdominal muscle fibre of fly adult. *D, E* Representative m.e.j.c. and g.e.j.c., respectively, and semilogarithmic plotting of decay phases of m.e.j.c. *(D)* and g.e.j.c *(E)* before (■) and 15 min after (▲) application of 0.4 M ethanol, expressed as percentage of the maximum amplitude. The mean value of 20 currents from the same fibre were used. $\tau$ was calculated from the exponential part of the curves and following values were obtained: m.e.j.c=2.80 msec; g.e.j.c.=2.89 msec; after ethanol m.e.j.c.=1.59 msec, g.e.j.c.=1.61 msec. Holding potential=−75 mV, temperature=22 °C.

found to be almost identical for both giant and miniature e.j.c.s (figure 1, D, E), one can postulate that the decay of small uniquantal and large multiquantal synaptic currents at this junction is determined by the same general limiting factor, probably by the mean life-time of ionic channels. The generalized prolongation of the multiquantal currents revealed by comparison of the $t^{dec}_{1/2}$ value is well-explained by asynchronous release of individual quanta, which leads to a time shift at the beginning of the exponential part of the decay curve.

Voltage and temperature dependence was studied on a group of fast m.e.j.c.s. It was found that the rise time and the decay time are prolonged slightly but significantly (t-test, $p \leqslant 0.01$) with hyperpolarization ($t^{rise}$ at $-50$ mV and $-110$ mV $= 1.60 \pm 0.04$ msec and $1.77 \pm 0.04$ msec, respectively; $t^{dec}_{1/2}$ at $-50$ mV and $-120$ mV $= 3.11 + 0.13$ msec and $3.96 \pm 0.3$ msec, respectively; figure 2, $n = 17$, temperature $= 22$ °C). Similar values were obtained from 2 fibres with multiquantal spontaneous e.j.c.s. The voltage dependence of the time course of *Drosophila* larval m.e.j.c.s therefore has the opposite direction to that found under similar conditions in locust muscles[2] (see also Onodera and Takeuchi[9,10], but Anwyl and Usherwood[11]). On the other hand, changes in temperature affected the time course of m.e.j.c.s in a similar way to locust muscles[2]. Figure 2 demonstrates the prolongation of $t^{dec}_{1/2}$ with the decrease in temperature, $Q_{10}$ being 3.82 between 31 and 22 °C and 1.33 between 22 and 10 °C, respectively. The temperature dependence is nonlinear, similarly as at the locust neuromuscular junction[12]; however $Q_{10}$ of *Drosophila* currents is decreased at lower temperature.

From the studies of neuromuscular junctions in vertebrates, it is known that many chemicals modify the time course of end plate potentials and ionic currents at the cholinergic postjunctional membrane[3]. Some of them have also been tested on fly muscles fibres: atropine[13,14] $2.10^{-4}$ M, scopolamine[15] $2.10^{-4}$ M, lidocaine[16,17] $2.10^{-4}$ M, serotonin[18] $10^{-4}$ M QX-222[16,17] $2.10^{-4}$ M and ethanol[19] 0.4 M. Except for ethanol, none of these drugs when added to the muscle bath affected either the amplitude or the time course of e.j.c.s (t-test applied in all cases, $p > 0.90$). The only effective drug, ethanol, shortened the time course of e.j.c.s, (see also Adams et al.[19]) i.e. it acted in exactly the opposite manner to that at the vertebrate cholinergic junction[20]. The rise time of e.j.c.s of ethanol treated preparations was shortened by 27.5% ($t^{rise} = 1.16 \pm 0.03$ msec, $n = 10$, MP $= -50$ mV) and $t^{dec}_{1/2}$ ($1.41 \pm 0.03$ msec, $n = 10$ MP $= -50$ mV, figure 2) decreased even more markedly, namely by 54%. A similar result was obtained on 1 fibre with multiquantal e.j.c.s (figure 1, E). In this figure (figure 1, D, E), a semilogarithmic plot of the decay phases of control e.j.c.s was made and the effect of ethanol on $\tau$ was estimated (see text to figure 1). In both cases $\tau$ decreased by 45%, indicating that ethanol reduced the time course of both uni- and multiquantal responses in the same way, apparently by shortening the ionic channel life-time. Parallel to this, the peak amplitude was reduced by 24%. A number of factors could contribute to this decrease, the most likely being a reduction of single channel conductance. Other possibilities should also be considered, such as systematic underestimation of the amplitude of extremely fast m.e.j.c.s under the conditions of recording, over a limited frequency range (3–5 kHz).

The findings presented here, with respect to properties of the ionic currents in glutamate-sensitive *Drosophila* muscles, are in good general agreement with similar data obtained in the locust with respect to the temperature dependence[2]. On the other hand, the polarity of the potential dependence was found to be opposite to that in the locust preparation. It is obvious that the dipole model of the transmitter-receptor channel complex[21] is also suitable for a formal interpretation of e.j.c.s time course in the fly. In this case, the direction of the dipole component of the complex should have the same polarity as that of acetylcholine receptors in vertebrate muscle. However, the sensitivity of the dipole to changes of electric field is smaller in the case of the *Drosophila* postjunctional membrane, and the pharmacological properties of activated ionic channels of these 2 membranes are different.

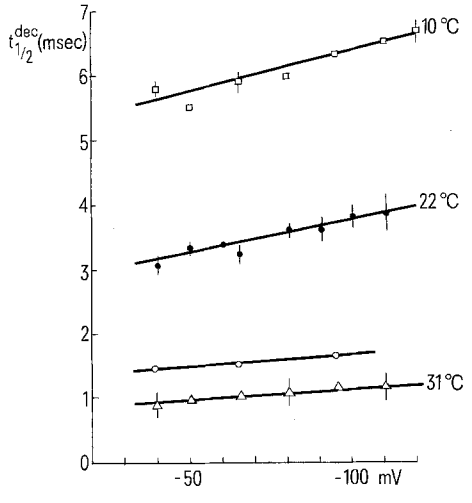


Fig. 2. Temperature and voltage dependence of the half decay time ($t^{dec}_{1/2}$) of the fast spontaneous e.j.c.s. 6–17 muscle fibres were examined at 3 temperatures (□: 10 °C, ●: 22 °C and △: 31 °C) and at least 30 e.j.c.s in each fibre were measured from photographic records. Each point represents the average value from all fibres. In several cases the standard error of the mean is smaller than the symbol. ○: $t^{dec}_{1/2}$ after 15 min of presence 0.4 M ethanol, at 22 °C (10 fibres).

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