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TIME INTEGRAL OF SYNAPTIC CONDUCTANCE

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SUMMARY

1. Inhibitory post-synaptic currents (i.p.s.c.s) were recorded under voltage clamp from neurones of *Aplysia* buccal ganglia.

2. The synaptic charge, Q, transferred by each i.p.s.c. was calculated as the time integral of the synaptic current, approximated by numerical integration. For typical i.p.s.c.s recorded at or near resting potential, $Q = -100$ to -500 pC. The majority of the charge is transferred during the interval between the peak and a time one time constant later.

3. In order to characterize an alternative measurement of synaptic efficacy, the slope of the Q *vs.* membrane potential curve was calculated and defined as the time integral of conductance, b. Values of b ranged from 2·6 to 51 pC/mV, averaging 14 pC/mV. For i.p.s.c.s recorded in thirty-one cells at room temperature, b was well correlated with G_{peak}, the peak synaptic conductance ($r = 0{\cdot}86$).

4. In most synapses, the time integral of conductance, which incorporates both amplitude and duration, may be a more revealing measure of synaptic efficacy than peak conductance.

5. Size of synaptic response was determined as a function of temperature, T. While G_{peak} decreases with decreasing temperature over the range 9–22 °C, b peaks at 12–18 °C and decreases at higher and lower values of T. The data permit the speculation that lengthening average channel lifetime, and therefore time constant of decay, with decreasing temperature, may have adaptive significance in maintaining synaptic efficacy.

INTRODUCTION

Most studies in synaptic physiology have used the peak membrane voltage or current change due to a post-synaptic potential (p.s.p.) or current (p.s.c.) as a convenient measurement of the size of the response. Duration of p.s.p. or p.s.c. has also been measured, and again has been used both as an aid to understanding mechanisms and also as a measurement of synaptic effectiveness. An alternative way to measure the size of a synaptic response is to combine duration with amplitude, in the form of the time integral of a synaptic voltage or current change. This technique, however, has been used only intermittently, and most often as an occasional adjunct to more conventional measurements of peak amplitude (Fatt & Katz, 1951; Takeuchi & Takeuchi, 1959; Rinzel & Rall, 1974; Barrett & Crill, 1974; Dudel, 1975). Rall (1959) and Calvin (1969) each suggested that p.s.p. area might be a more

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important measure of synaptic strength than the usual height, and Barrett & Crill (1974) defined synaptic effectiveness in terms of the time integral of p.s.p. in cat motoneurones. However, no systematic comparison of peak value to time integral has been made for post-synaptic currents or potentials, nor has a time integral measurement analogous to peak conductance been examined.

Recording from inhibitory synapses of *Aplysia* buccal ganglia (Gardner & Kandel, 1977; Gardner & Stevens, 1980; Gardner, 1980), measurements have been made of peak synaptic current and its time integral, synaptic charge transfer, and have been used to derive a time integral measure of synaptic conductance. The time integral of conductance is compared to peak conductance under conditions in which the decay time constant of the post-synaptic current is unaltered, as well as under conditions of changing temperature, in which both peak synaptic amplitude and decay time constant vary. The data to be presented indicate that time integral of conductance may be a more valid measure of synaptic efficacy than peak conductance, and is especially appropriate where charge transfer is of interest.

A short report of these findings has been presented to the Biophysical Society (Gardner, 1979).

METHODS

Recording. Storage of experimental animals, dissection of ganglia, preparation of electrodes, recording, voltage-clamping and data acquisition have been previously described (Gardner & Stevens, 1980; Gardner, 1980). Inhibitory post-synaptic currents evoked by stimulation of identified presynaptic neurones were recorded under voltage clamp from cells of the buccal ganglia of *Aplysia californica*. Temperatures were measured with thermistor probes (Yellow Springs Instruments) and recorded with 0·2 °C precision. Two particular techniques deserve emphasis: (1) aside from those used in a few preliminary studies, all micro-electrodes were bevelled to yield resistances of less than 6 MΩ; this procedure ensured stable base lines for current records and low-noise tails: (2) currents were filtered with a four-pole active filter with true Bessel characteristics (Ithaco 4212) in order to achieve maximum noise reduction without distorting the shape of the current transient.

Analysis and integration. Current and voltage transients were digitally sampled and recorded on digital magnetic tape, then recalled for subsequent analysis. Peak currents were measured for all cells in this study as described in the previous paper. Only cells in which the peak synaptic conductance exceeded 0·1 μmho were used to compute synaptic charge transfer, as smaller peak conductances produced current transients in which the noise in the tails contributed significantly to the computed area. This restriction meant eliminating nine cells out of sixty-five. Individual current transients in which i.p.s.p.s gave rise to uncontrolled spikes (see Gardner, 1979), were discarded, as were records showing any other irregularity such as contamination from additional synaptic input on the decay phase of the i.p.s.c.

Synaptic current traces were displayed on the computer oscilloscope and a zero current base line was matched by eye to the level of current either preceding, or 220–1100 msec following, the peak of the i.p.s.c. No smoothing was employed. Currents were integrated numerically over time by scanning with a Δt equal to the original sampling interval. Limits of integration were set as cursors overlying each displayed trace in order to avoid including stimulus artifact currents in the integral. In addition, the later cursor was set about three time constants beyond the peak so as to minimize contributions to the integral from small fluctuations of current near the base line. This procedure results in underestimating the charge in the current tail by an error of less than 5%. The result of this integration process is a value for synaptic charge transfer. These changes in membrane charge due to synaptic currents were then plotted as a function of membrane potential to produce synaptic charge *vs.* voltage (Q–V) curves in a manner analogous to that used to produce synaptic peak current *vs.* voltage (I–V) curves.

RESULTS

Measurement of synaptic charge transfer

In an attempt to find a parameter of synaptic efficacy which incorporates duration as well as peak amplitude, I have computed the time integral of the synaptic current, as illustrated in Fig. 1. The synaptic charge, Q, is defined as

$$Q = -\int_0^\infty I(t)\,dt, \tag{1}$$

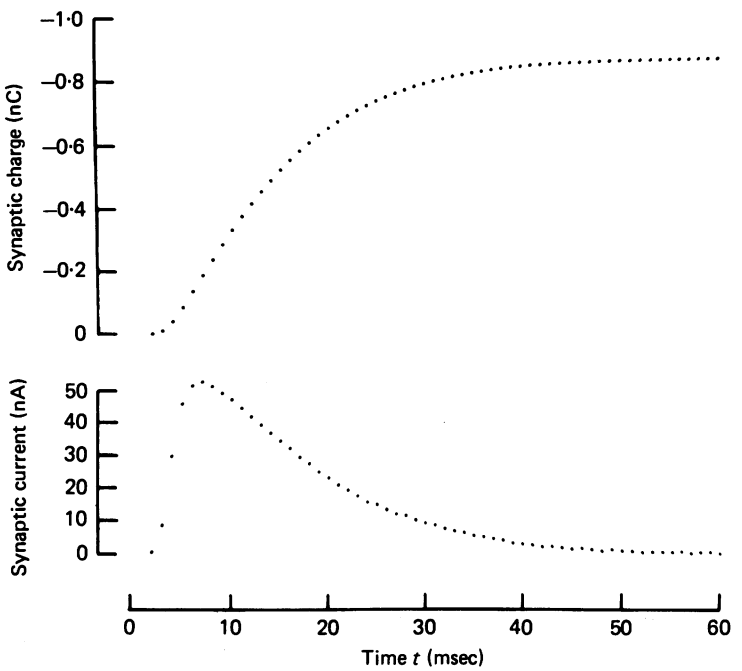

Fig. 1. Time integration of synaptic current. Lower curve, synaptic current, I, *vs.* time, t; an i.p.s.c. recorded at $V_m = -24$ mV and digitized by sampling at 1 msec intervals. Upper curve, synaptic charge, Q, *vs.* time; the time integral of I obtained by numerical integration of the lower curve with $\Delta t = 1$ msec, coinciding with the sample interval for convenience. Both current and charge curves are plotted on a common time axis.

where $I(t)$ describes the synaptic current at time t. Since current was not recorded as a continuous function of time, but was instead sampled at 1 msec or greater intervals and stored, the time integral was approximated by numerical integration,

$$Q = -\sum_{t=0}^{3\tau} I(t)\Delta t, \tag{2}$$

with Δt equal to the sampling interval and τ = time constant of i.p.s.c. decay. The lower trace in Fig. 1 shows an inhibitory post-synaptic current (i.p.s.c.) recorded by sampling at $\Delta t = 1$ msec. Each point in the lower trace determines the height of a rectangle of width Δt; each point in the upper trace is the sum of all past rectangles and shows the sum of the charge transferred up to that time by the

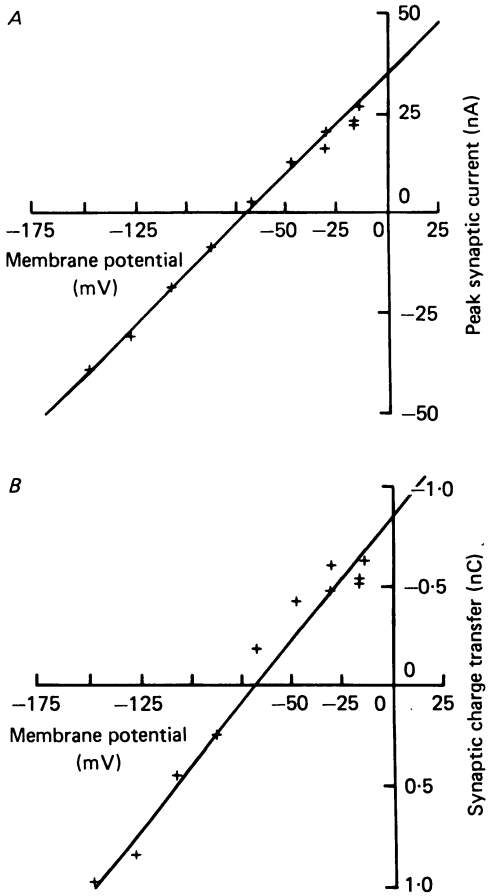

Fig. 2. Synaptic current *vs.* voltage and synaptic charge *vs.* voltage. *A*, current–voltage (*I–V*) curve obtained by plotting (+) the peak value of each of eleven synaptic currents, I_{peak}, recorded at various V_m values *vs.* V_m. *B*, charge–voltage (*Q–V*) curve obtained similarly by plotting (+) synaptic charge transfer, *Q*, for the same eleven currents *vs.* V_m. In both *A* and *B*, a least-squares line has been fitted to the data points more hyperpolarized than the synaptic reversal potential. In *A*, the line is described by $I_{peak} = G_{peak}(V_m - E_{i.p.s.c.})$ with $G_{peak} = 0{\cdot}50$ μhmo and $E_{i.p.s.c.} = -70$ mV; in *B*, the line is $Q = -b(V_m - E_{i.p.s.c.})$ with $b = 12{\cdot}5$ pC/mV and $E_{i.p.s.c.} = -70$ mV (note sign convention). Least-squares linear fits of the *I–V* and *Q–V* data are determined only from values of *I* and *Q* recorded at V_m more hyperpolarized than the reversal potential, because at depolarized values of V_m the recorded duration of the i.p.s.c. is shortened by activation of non-synaptic currents (Gardner, 1980).

current shown in the lower trace. Because of the smoothly varying shape of the i.p.s.c. as a function of time, and the small Δt, smoothing by Simpson's or the trapezoidal rule was not employed. In order to minimize errors from noise in long tail currents near the base-line level, the upper limit of integration was set at the approximate point the i.p.s.c. noise tangentially approached base line. In no case was this limit closer than three times the decay time constant of the i.p.s.c.

The integral obtained in this way is thus the effective charge, *Q*, transferred by post-synaptic current flow, with the sign of *Q* defined to agree with the sign of the

net charge acquired by the cell as a result of the synaptic current. Positive Q thus means acquisition of positive charge or loss of negative charge. Because inward currents, which are by convention negative, lead to acquisition of positive charge, it has been necessary to introduce a negative sign in eqns. (1) and (2).

Like peak current, synaptic charge transfer can be measured with good repeatability. For these inhibitory synapses, Q is negative at the resting potential and

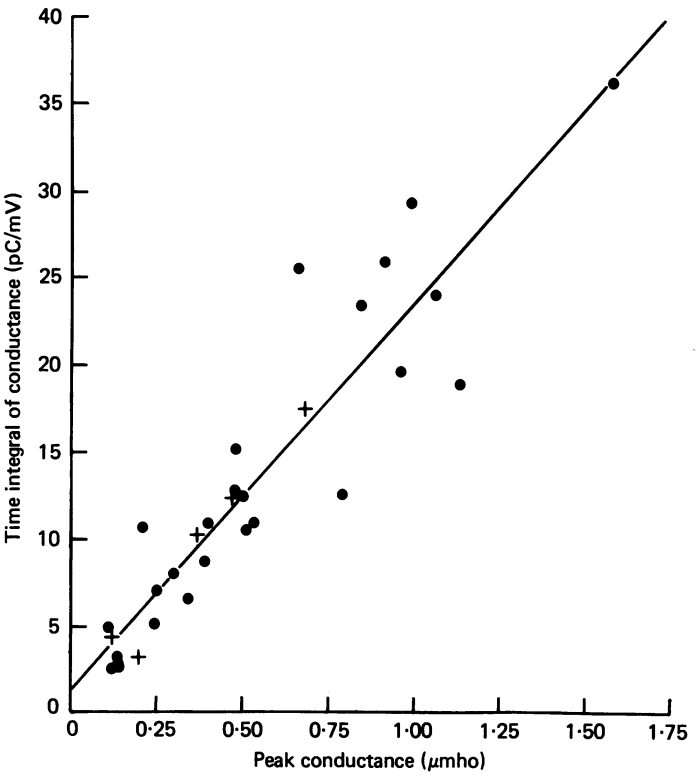

Fig. 3. Time integral of conductance *vs.* peak conductance. For each of twenty-six cells (●) bathed in 60 mM-Ca sea water and each of five cells (+) bathed in 80 mM-Ca and 144 mM-Mg sea water, the time integral of synaptic conductance b is plotted *vs.* peak synaptic conductance G_{peak}. All measurements at room temperature (21 ± 2 °C). The least-squares line fitting the thirty-one data points is described by b (pC/mV) = 22·5 msec. G_{peak} (μmho) + 1·23 pC/mV, with correlation coefficient r = 0·86.

reverses with hyperpolarization to larger and larger positive values. Maximum charge transfers at these synapses were recorded at membrane potentials 70–90 mV hyperpolarized from reversal, and reached 2000 pC for synapses with peak conductances of about 1 μmho. In a more physiological range of membrane potentials, 5–20 mV depolarized from equilibrium, −100 to −500 pC are transferred by these same synapses.

The upper curve of Fig. 1 additionally shows that the majority of the charge is transferred during the interval between the peak and one time constant past the peak, with rising phase and tail contributing only a small fraction of the total charge.

Time integral of conductance

Fig. 2*A* displays the synaptic *I–V* curve of a set of i.p.s.c.s. Values of synaptic charge transfer recorded from the same i.p.s.c.s are plotted *vs.* membrane potential in an analogous manner to produce the synaptic *Q–V* curve (Fig. 2*B*). For voltages near the reversal potential, where the peak current is small, fluctuations in the

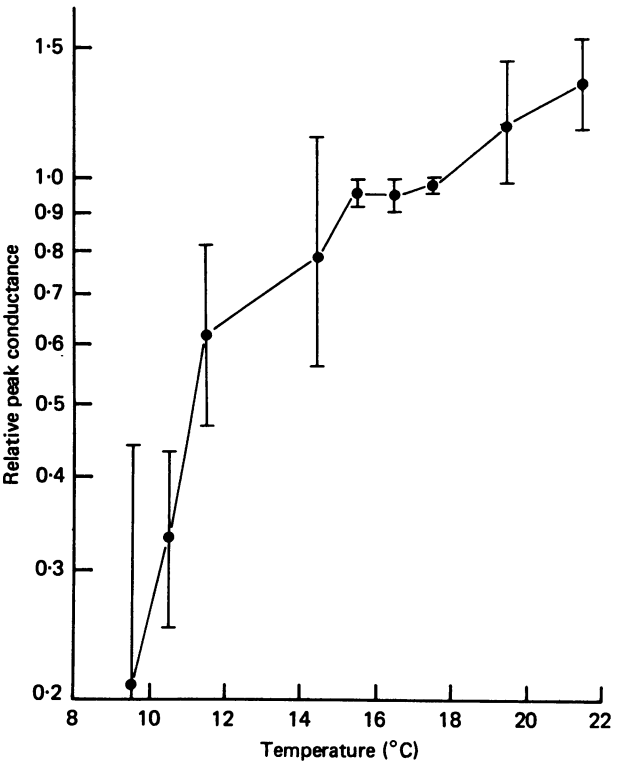

Fig. 4. Relative peak synaptic conductance *vs.* temperature. Data from nine cells. For each cell, peak synaptic conductances, G_{peak}, at several temperatures, T, were determined by the method of Gardner & Stevens (1980). Values of peak conductance at each temperature were normalized by dividing each by the peak conductance at T = 16° or 17 °C. For each 1° range, values of these normalized G_{peak} were averaged and plotted, with S.E. bars, as a single point (●) in the centre of the range. For example, all normalized G_{peak} values measured for $21° \leqslant T < 22$ °C were averaged and plotted at $T = 21{\cdot}5$ °C.

recorded current seem to affect the integral more than the peak, producing greater uncertainty to charge measurements. For this reason, values of reversal potential obtained by interpolation are more easily determined from *I–V* than from *Q–V* curves.

The slope of the least-squares line fitted to the *Q–V* curve is defined as the time integral of conductance *b*, by analogy to the definition of peak conductance, and is given convenient units of pC/mV or, equivalently, μmho-sec. In thirty-two neurones examined, *b* ranged from 2·6 to 51 pC/mV, with an average of 14 ± 2 (S.E.) pC/mV.

Independent measurements of peak conductance, time integral of conductance

and decay time constant can be used to compare recorded i.p.s.c.s to the predicted behaviour of instantaneously rising synaptic events. For example, Q and b were measured for series of i.p.s.c.s recorded at 21 ± 2 °C, and b plotted *vs.* G_{peak} (Fig. 3). The least-squares line fitted to this summary plot has a slope $\Delta b/\Delta G_{peak}$ of 22·5 msec, while the average time constant of decay, τ, for this group of cells was $20{\cdot}3 \pm 0{\cdot}9$ msec.

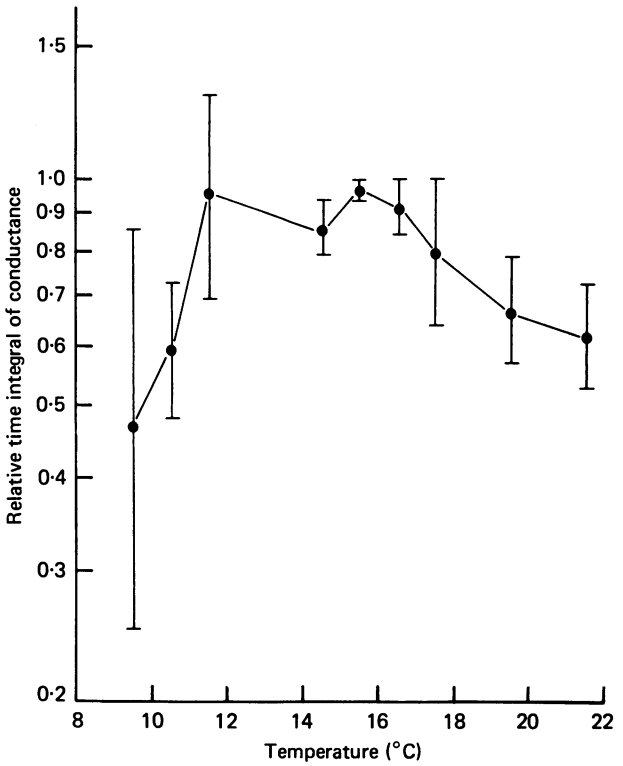

Fig. 5. Relative time integral of conductance *vs.* temperature. This graph was obtained by a process analogous to that used to produce Fig. 4 using data from the same cells, except that time integral of conductance b, rather than G_{peak}, is used here as the relative measure of synaptic efficacy.

The $\Delta b/\Delta G_{peak}$ slope is thus about 10% greater than would be predicted for an instantaneously rising i.p.s.c. without broad peak. The correlation between time integral of conductance and other methods of determining synaptic amplitude is even better if b is compared not to G_{peak} alone, but to the product of peak conductance and time constant of decay, $G_{peak}.\tau$. When τ, G_{peak}, and b were each determined independently for twenty-five cells, the best linear fit,

$$b = 1{\cdot}26.\tau.G_{peak} + 0{\cdot}03 \text{ pC/mV} \quad (3)$$

had a high correlation coefficient ($r = 0{\cdot}98$). The slope of 1·26 indicates that about 20% of the charge is transferred during the rising phase and the broad peak.

Changes in synaptic efficacy with temperature

Cooling the *Aplysia* buccal ganglia lowers the peak amplitude and prolongs the decay of the synaptic conductance change. The increase in the time constant of decay is exponential over the temperature range 22–9 °C, with an average Q_{10} of 5, suggesting that i.p.s.c. channel lifetime is prolonged by cooling (Gardner & Stevens, 1980). Time integral of conductance may be a more useful measure than peak conductance for evaluating variables, such as changes in temperature, which affect both amplitude and duration of synaptic currents. Figs. 4 and 5 compare the two measures. In Fig. 4, peak conductances at each of several temperatures were normalized to their values at 16 or 17 °C for each of nine cells, and the averaged values plotted as a function of temperature. The peak conductance *vs.* temperature relation shows that cooling from 22° to 9 °C produces a progressive decrease in peak synaptic conductance. Unlike decay time constant, which increases exponentially with cooling over the same temperature range, peak conductance does not depend upon temperature in any simply characterizable way.

In Fig. 5, the normalized time integrals of conductance, b, from the same sets of i.p.s.c.s are also plotted as a function of temperature. Unlike G_{peak}, b does not decrease with cooling; instead, b is constant over the 12–18 °C temperature range, declining for $T < 12$ °C and $T > 18$ °C. The average value of b for $T < 12°$ and that for $T > 19°$ are each significantly smaller than b at 14–18 °C ($P < 0{\cdot}05$).

DISCUSSION

Comparison of peak and time integral as measures of synaptic response

The size of a post-synaptic response may be measured by the peak amplitude of the response, such as peak voltage change, peak current flow or peak conductance, or by their respective time integrals, such as the p.s.p. time integral, synaptic charge transfer, or time integral of conductance. When synaptic potential is measured, peak values may be preferred to time integrals, or vice versa, under different conditions. For example, Rall (1959) suggested for excitatory synapses that the area of synaptic transients was of more importance for reaching threshold than the peak amplitude of individual events. However, Barrett & Crill (1974) point out that area of a potential becomes a more useful measure only where summation is necessary; for large excitatory post-synaptic potentials where the peak exceeds threshold, the peak value is of greater importance.

When synaptic currents or conductance changes are measured, a preference for peak or time integral measurements must follow consideration of which parameters determine p.s.p. height and which determine p.s.p. area. Full analyses require knowledge of synaptic distance and passive membrane properties, and involve calculation of convolution integrals of the synaptic conductance as a function of time, and the membrane pulse response (Jack, Noble & Tsien, 1975). However, two simplified cases may be considered. Where the membrane time constant is slower than, or of comparable value to p.s.c. decay, both the peak and time integrals of the p.s.p. will depend primarily on the time integral of the synaptic conductance, rather than on its peak value. Thus whether peak potential change or p.s.p. area is

of interest, time integral of conductance provides a more useful measure of synaptic effectiveness than peak conductance. If the membrane time constant is much faster than p.s.c. duration, then the peak p.s.p. amplitude will be determined by the peak conductance, and p.s.p. area by the time integral of conductance. For this case, only where p.s.p. peak amplitude is of particular interest will peak conductance be preferable to its time integral.

There remain many advantages to the use of peak conductance, or corresponding voltage or current measurements, as indicators of synaptic amplitude. Peak measurements are easily made in the presence of additional synaptic input, slow base-line drift, or noise. Since the time to peak of most post-synaptic events is relatively fast when compared to the decay, the time interval required to be free from noise or drift is much shorter when peak amplitudes are to be measured, rather than time integrals of the entire event. I have also found peak current to be a more useful indicator of synaptic reversal potential, in spite of the suggestion of Calvin (1969) that the use of time integral might be indicated here.

Measurements of peak value and of time constant may provide an acceptable estimate of time integral of conductance, since time integral was found to be a linear function of the product of peak conductance and of decay time constant. Finally, changes in measured peak values alone may be good indicators of changes in time integral for certain uses. For example, peak conductance measurements are a valid metric for dose–response studies of drugs which do not affect time course.

Comparison to the unitary event

The time integral of conductance of the i.p.s.c. had an average value of 14 pC/mV at 21 °C, a value which may be compared to the conductance time integral of elementary channel events at these *Aplysia* synapses. Gardner & Stevens (1980) identified their measured i.p.s.c. decay time constant with elementary channel lifetime, and also calculated upper estimates for single-channel conductance, both in these same *Aplysia* neurones. From their data, a two-state channel with 20 msec average lifetime and 10 pS open conductance would have a time integral of conductance of 2×10^{-4} pC/mV. Combining this value with that for the nerve-activated i.p.s.c. suggests that some 60,000–80,000 individual channel openings comprise the macroscopic synaptic event. This value is likely to be an underestimate, as it assumes that the synaptic charge used to compute b is in fact the total synaptic charge delivered at the synaptic site (Q_0 of Barrett & Crill, 1974). For synapses such as these, which are remote from the recording and clamp electrodes in the soma, some charge loss cannot be ruled out. At other synapses, values for the time integral of the elementary conductance which may be calculated from published values of conductance and duration are comparable to those reported here.

Temperature effects on size of synaptic response

Studies of synaptic function in poikilotherms have found decreases in peak synaptic current or voltage with decreasing temperature over the range 9–25 °C. For example, cooling produces a decrease in peak synaptic voltage (Eccles, Katz & Kuffler, 1941) and current (Gage & McBurney, 1975) at amphibian neuromuscular junction and in the mollusc *Planorbarius* (Ger & Zeimal, 1977), as well as a decrease in single-

channel conductance of locust neuromuscular junction (Anderson, Cull-Candy & Miledi, 1977). For *Aplysia* acclimated to habitats at 14–17 °C and kept at these temperatures in the laboratory prior to experimentation, synaptic function as measured by peak amplitude varies with temperature in the same way. Peak current increases with warming from the temperature of acclimation, and decreases with cooling. Although alternative hypotheses are available, it can be argued that adaptation in a poikilotherm might require either most effective synaptic function at the temperature of acclimation, or else temperature-invariant function over a wide range.

The use of time integral of conductance rather than peak provides an alternative way to interpret the effects of temperature on synaptic amplitude. In contrast to the peak, the time integral of conductance showed a maximum between 12 and 18 °C, falling off at both higher and lower temperatures (Fig. 5). Even if these data are viewed as showing no great change in time integral of conductance with temperature, this should not be interpreted to mean that synaptic charge at a given voltage is a constant of the system which is distributed in time by temperature-dependent factors. Channel lifetime, which determines the decay time constant of i.p.s.c.s, increases with cooling (Gardner & Stevens, 1980), while transmitter release and channel-opening rate, which determine i.p.s.c. peak amplitude, both decrease with cooling. Maintaining a constant charge transfer, or time integral of conductance, with changing temperature would therefore be the result of separate temperature-dependent effects in a number of independent processes. It is possible that the high Q_{10} of synaptic channel lifetime found here and at other synapses may have adaptive significance as a mechanism to maintain synaptic function with varying temperature.

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