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An evaluation of causes for unreliability of synaptic transmission

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ABSTRACT **Transmission at individual synaptic contacts on CA1 hippocampal pyramidal neurons has been found to be very unreliable, with greater than half of the arriving presynaptic nerve impulses failing to evoke a postsynaptic response. This conclusion has been reached using the method of minimal stimulation of Schaffer collaterals and whole cell recording in hippocampal slices; with minimal stimulation only one or a few synapses are activated on the target neuron and the behavior of individual synapses can be examined. Four sources for the unreliability of synaptic transmission have been investigated: (*i*) the fluctuation of axon thresholds at the site of stimulation causing the failure to generate a nerve impulse in the appropriate Schaffer collaterals, (*ii*) the failure of nerve impulses generated at the site of stimulation to arrive at the synapse because of conduction failures at axon branch points, (*iii*) an artifactual synaptic unreliability due to performing experiments *in vitro* at temperatures well below the normal mammalian body temperature, and (*iv*) transmission failures due to probabilistic release mechanisms at synapses with a very low capacity to release transmitter. We eliminate the first three causes as significant contributions and conclude that probabilistic release mechanisms at low capacity synapses are the main cause of unreliability of synaptic transmission.**

Several recent studies have documented the unreliability of central nervous system synapses: typically, a postsynaptic response is produced less than half of the time when a presynaptic nerve impulse arrives at a synapse (1–3). Synaptic unreliability, defined as the fraction of presynaptic impulses that fail to cause a postsynaptic response, has been thought to result from the statistical nature of neurotransmitter release (1–3). But other sources for the unreliability, described below, have not been excluded.

Four alternative causes for synaptic unreliability need to be considered: (*i*) Threshold fluctuations. If the threshold of axons being stimulated happened to fluctuate randomly from stimulus to stimulus (4, 5) and if the stimulating current is near threshold, the apparent failures in transmission might reflect those trials on which the stimulus failed to reach threshold. According to this mechanism, synaptic transmission would appear to fail simply because no action potential had been generated at the stimulation site. (*ii*) Conduction failures. Perhaps action potentials that are generated at the site of the stimulating electrodes fail to propagate to some terminal branches of the axon. Were this the case, then synaptic transmission might appear to fail because the action potential never reached the axon terminal. (*iii*) Temperature. Transmitter release actually might be very unreliable at the low temperatures (around 24°C) typical of most slice and culture experiments, but quite reliable at normal body temperature. Were this the case, the unreliability would just be an experimental artifact without computational implications for normal brain function. (*iv*) Probabilistic transmitter release. Synapses have long been known to release neurotransmitter probabilistically (6). If the quantal content is small (because the number of release sites is small and/or release probability per site is low), synaptic transmission would be expected to be unreliable. This has been the explanation favored by earlier workers (1–3).

We have investigated these causes for unreliability at excitatory synapses on pyramidal neurons of the CA1 region of hippocampus in brain slices and conclude that synaptic unreliability does, as previously assumed, arise from the probabilistic release processes. Further, we find that reliability does not differ appreciably between room temperature and 35°C, so that conclusions about synaptic reliability from experiments at room temperature apply to functioning of synapses at body temperature.

After describing the general approach of the experiments, we consider these possible mechanisms for the unreliability of synaptic transmission in turn.

METHODS

We have used standard methods as described by Stevens and Wang (7). Briefly, transverse hippocampal slices were prepared from Harlan–Sprague–Dawley rats between 2 and 4 weeks old. After an hour of stabilization in an incubation chamber, slices were mounted submerged in the recording chamber and superfused with a 95% O_2/5% CO_2 solution containing 120 mM NaCl, 3.5 mM KCl, 1.25 mM NaH_2PO_4, 26 mM $NaHCO_3$, 1.3 mM $MgCl_2$, and 2.5 mM $CaCl_2$; Mg and Ca concentrations were modified in some experiments as specified in the text. Whole cell voltage clamp recordings were obtained from CA1 (or CA3 as specified in the text) pyramidal cells using 3- to 5-MΩ pipettes filled with a solution containing 130 mM cesium gluconate, 5 mM CsCl, 1 mM $MgCl_2$, 2 mM MgATP, 0.2 mM GTP, 5 mM NaCl, and 10 mM Hepes (pH 7.25). The Schaffer collateral pathway was stimulated (tungsten bipolar electrodes) with 0.1-ms duration pulses at a rate of 0.1 Hz. Stimulus intensity was varied systematically and a minimum of 32 records were collected at each stimulus intensity near those values that produced a significant number of failures in synaptic transmission. After the experiment, each trace was scrutinized and postsynaptic responses were classified as a failure or success visually.

RESULTS

Unreliability Estimated by Minimal Stimulation. We have estimated synaptic unreliability with the method of minimal stimulation (1). The idea of minimal stimulation is to decrease the stimulation current until only a single axon (that projects to the neuron from which recordings are being made) is activated. This situation is considered to occur when the average postsynaptic current amplitude and failure probability remain constant over a range of stimulus intensities just near the threshold for detecting a response. An example of data from such an experiment is illustrated in Fig. 1. In this figure, the probability of a failure in synaptic transmission

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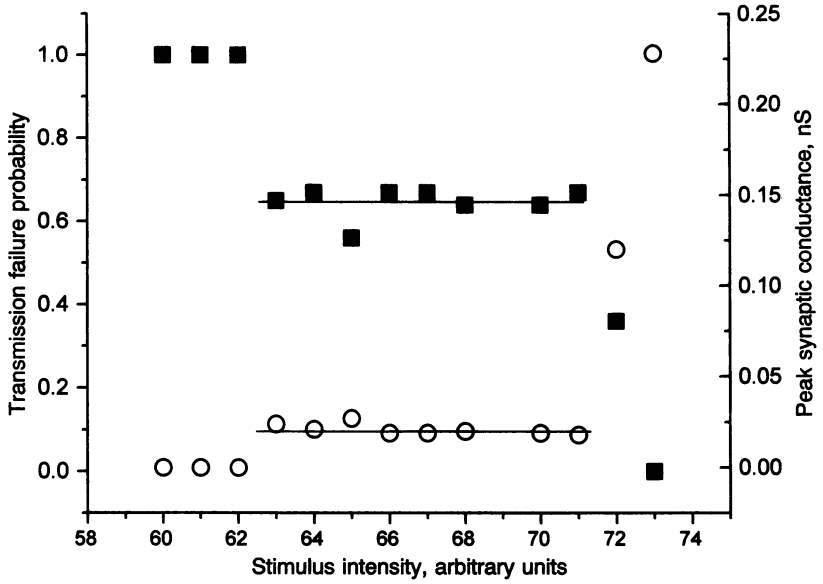

FIG. 1. Estimate of synaptic unreliability (fraction of stimulation trials that failed to result in neurotransmitter release) with the minimal stimulation method. Stimuli are applied to the Schaffer collateral pathway and synaptic currents are recorded (whole cell voltage clamp) from a CA1 pyramidal cell. Open circles give the average peak synaptic conductance (right ordinate) and solid squares specify the probability that synaptic transmission does not occur (left ordinate), both as a function of stimulus intensity in arbitrary units. Note that both the failure probability and mean peak conductance remained essentially unchanged as the stimulus intensity was varied over an ≈12% range (from 63 to 71 units). The estimate of synaptic unreliability would be taken as the fraction of transmission failures (0.65) that remained constant as stimulus intensity varied. As the stimulus intensity is increased beyond 71, other axons projecting to the target cell are recruited and the peak response size increases rapidly as the rate at which transmission failures approach 0.

was near 1 for the lowest stimulus intensities and then decreased to a plateau around 0.65; for a relatively wide range of stimulus intensities, the probability of evoking a postsynaptic current remained constant as stimulus intensity was increased. Over this same range of stimulus intensities, the average peak synaptic conductance also remained unchanged in the face of increasingly larger stimuli. In an ideal situation, this plateau would correspond to the activation of only a single fiber that projects to the postsynaptic neuron from which we were recording, and the transmission failure rate would be taken as the unreliability of that synapse. Of course, sometimes several axons projecting to the test neuron may have very similar thresholds. For this reason, the minimal stimulation method gives an estimate only for the lower limit on synaptic unreliability. That is, the actual unreliability would be greater than the apparent unreliability on those occasions when more than a single axon was being activated.

We find, in confirmation of what has been reported earlier (1–3), that unreliability, estimated as for the cell in Fig. 1, usually is greater than 0.5. The mean failure rate for synaptic transmission in 21 synapses studied at room temperature was found to be 0.71, with a range from 0.3 to 0.95. A histogram of the relative frequencies of the various probabilities for failure of synaptic transmission for individual neurons (presumably individual synapses) is presented in Fig. 2. Note that most failure rates seem to be represented by a broad peak between 0.5 and 0.95. Because synapses with very low reliability are difficult to detect, we suspect our sample could well be biased against the most unreliable synapses. Also, the synapses we studied might have been, for reasons noted above, less reliable than we found if we were frequently stimulating more than a single axon.

Synaptic Unreliability at Body Temperature. Is the unreliability of synaptic transmission described above an artifact of the low temperature at which these experiments were carried out? To answer this question, we have compared synaptic reliability measured at room temperature with that determined when the slice temperature is close to 35°C. For eight cells studied at 32–37°C, we have found the average failure rate to be 0.72, with a range of 0.45–0.90, as compared to a mean failure rate of 0.71, with a range of 0.3–0.95, for 21 cells at room temperature. In three cells, failure rate was determined at both the low and high temperatures and did not change significantly when the temperature was changed. Thus, the temperature coefficients of the various processes that contribute to failures in transmission are approximately the same so that synaptic reliability is not much changed by a 10°C shift in slice temperature.

Axon Threshold Fluctuations. Axon threshold is not fixed, but rather—as has been long appreciated (4, 5)—fluctuates randomly. Because synaptic reliability has been estimated by

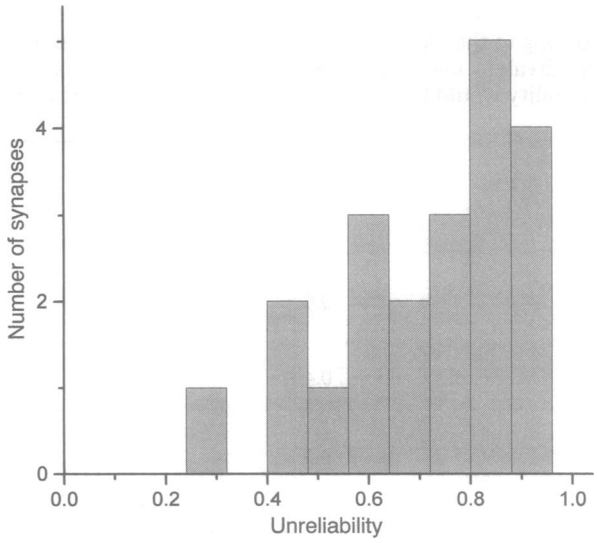

FIG. 2. Histogram of synaptic unreliability for 21 neurons measured at room temperature. For each case the stimulus was systematically varied and the highest failure rate for transmitter release that was constant over a range of stimulus intensities greater than about 5% was assigned to the neuron.

using electrical stimulation of fiber pathways to initiate presynaptic nerve impulses, some portion of the observed unreliability probably is due to axon threshold fluctuations: those axons near threshold will produce nerve impulses with a probability less than unity and, thus, will contribute to the apparent synaptic unreliability. This source of confounding variability is potentially important to all studies that use electrical stimulation to elicit nerve impulses but is of particular concern for studies that employ minimal stimulation, where the stimulus intensity is reduced to near threshold to activate only one or a few fibers that project to the neuron from which recordings are being made.

We have estimated the magnitude of axon threshold fluctuations in a straightforward manner: while applying near-threshold stimuli to Schaffer collateral pathway as in the experiments described above, we have recorded the probability with which antidromic action currents (whole cell recording) are elicited in a CA3 neuron. We find, as has long been known (4, 5), that the probability of an action potential being produced increases from 0 to 1 as the stimulus intensity is increased around threshold. This phenomenon is illustrated in Fig. 3, where the cumulative probability of an antidromic action current is plotted as a function of stimulus intensity, normalized to 100 at the intensity that produces an action current half of the time. The smooth curve in Fig. 3 is a cumulative gaussian function with a mean of 100 and a standard deviation of 2.7. In four experiments, we have estimated that the coefficient of variation of axonal threshold fluctuations is 2.8%, with a range of 2.0–3.5%. These values are very close to what has been reported for frog nerve (4, 5). As long as synaptic reliability remains approximately constant as the stimulus intensity is varied over a 5% range, threshold fluctuations should not be contributing significantly to estimates of synaptic reliability.

Conduction Failure and Probabilistic Release Mechanisms. The frequency of conduction failures is difficult to estimate because recording from presynaptic axons sufficiently close to the synaptic site being studied is not generally possible. Although less direct than we would like, we have used the following strategy to differentiate between conduction failure and probabilistic release of neurotransmitter. If the divalent ion concentration is maintained at a constant value while the ratio of calcium to magnesium concentrations is varied, then axon excitability (and thus conduction failure) should remain about constant, whereas release probability should vary according to the Dodge–Rahamimoff equation (8). Similarly, if the divalent ion concentration is increased, so that axon excitability would be decreased due to surface charge effects (9), conduction failures should consequently increase. But if the divalent concentration increase is made up by calcium ions, release failures should decrease. Fig. 4 demonstrates that synaptic transmission obeys the Dodge–Rahamimoff equation in all cases. Thus, with a constant divalent ion concentration but a variable ratio of calcium and magnesium concentrations, reliability increased and decreased according to the expectations of the equation. Further, transmission failures decreased as the calcium ion concentration increased, contrary to the expected effect on threshold through surface charge effects. We conclude that probabilistic release mechanisms, rather than conduction failures, explain synaptic unreliability.

DISCUSSION

We have confirmed that synaptic transmission at excitatory synapses is generally quite unreliable, with failure rates usually in excess of 0.5. Furthermore, we have demonstrated that these failure rates are not an artifact of the low temperatures at which *in vitro* experiments are typically carried out. Three sources for the unreliability have been evaluated. Although all three doubtless contribute to some extent, the dominant source is the stochastic nature of the neurotransmitter release processes. We could detect no evidence of conduction failures, and axonal threshold fluctuations contribute only for the fibers for which the stimulus intensity is within a few percent of the threshold. Experiments that employ minimal stimulation typically require that average response size, and transmission failure rate, not vary as the stimulus intensity is altered by more than 5% (see Fig. 1), so threshold fluctuations should not be an important contributor in such experiments.

Although we can conclude that the unreliability of central synaptic transmission is not an artifact of failure of nerve impulses to arrive at synapses or of the low temperatures usually employed in slice experiments, we have presented no evidence that bears directly on the unreliability of synaptic transmission *in vivo*. Neuromodulatory influences that are absent *in vitro* but present *in vivo* might, for example, increase synaptic reliability. This issue will have to be settled by direct estimates of synaptic reliability *in vivo*.

Both Rosenmund *et al.* (2) and Hessler *et al.* (3) argued for the existence of two populations of synapses, one with a reliability around 0.5 and the other with a reliability near 0.1. As Rosenmund *et al.* (2) pointed out, however, these earlier data might be consistent with many reliability classes, and not just two. Our data are inconclusive on the issue of how

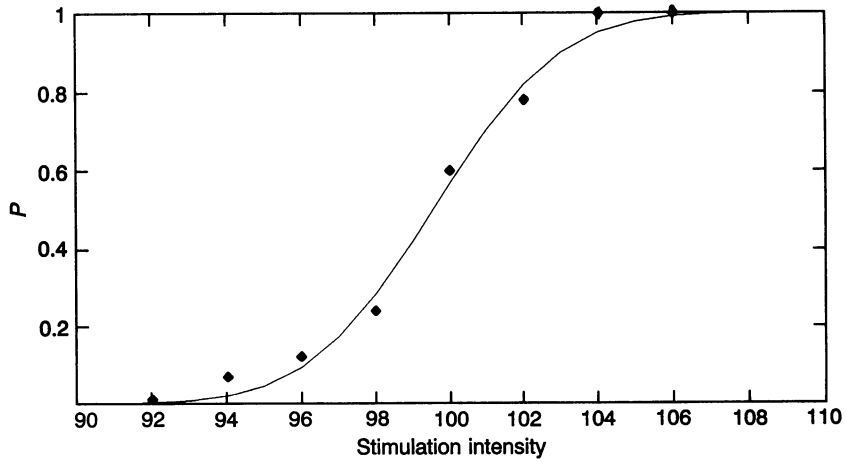

FIG. 3. Cumulative histogram of the probability of evoking an antidromic action current in a CA3 neuron as a function of stimulus intensity applied to the Schaffer collateral pathway. Data points give the observed cumulative relative frequency of an antidromic action current and the smooth line is a normal distribution fitted to the data with a mean normalized to 100 and a standard deviation of 2.7.

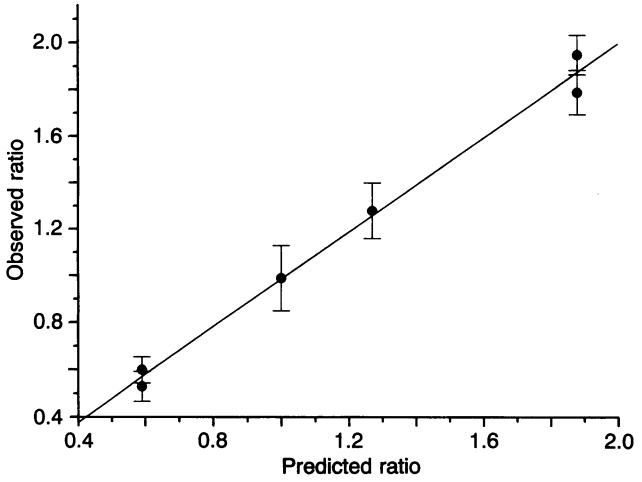

FIG. 4. Observed peak amplitude of average synaptic current, normalized to the amplitude in the standard solution (Mg concentration = 1.3 mM; Ca concentration = 2.5 mM), as a function of the ratio predicted by the Dodge–Rahamimoff equation (8): predicted ratio = $\{[1 + (1.3/K_2) + (2.5/K_1)][Ca]/2.5[1 + ([Mg]/K_2) + ([Ca]/K_1)]\}$, where [Ca] and [Mg] denote the calcium and magnesium concentrations in the test conditions, and $K_1 = 0.3$ mM and $K_2 = 1$ mM are fitted by a least squares procedure. The test calcium concentration ranged from 1.9 to 3.7 mM, and the test magnesium concentration ranged from 0.1 to 1.9 mM. For the largest and smallest ratios, the total divalent ion concentration was kept constant (3.8 mM), but the ratio of [Ca]/[Mg] was varied. For the predicted ratio of 1, the [Mg] was maintained at 1.3 mM and the [Ca] was increased to 3.4 mM.

many reliability classes are present. All unreliability values between 0.5 and 0.95 seem to be represented with a preponderance of the more unreliable synapses, but we cannot exclude the possibility that two classes predominate. In any case, the minimal stimulation method is not ideal for answering this question because sufficiently large sample sizes are difficult to acquire, the method places only a lower limit on unreliability, and the more unreliable synapses are probably underrepresented because they are difficult to detect. If the estimates from quantal analysis of release probabilities close to 1 for synapses on inhibitory neurons in hippocampus are accurate (10), mechanisms are available to provide release probabilities over the entire range of possibilities from very low to near certainty.

What are the implications of this synaptic unreliability for brain information processing? Since the majority of impulse arrivals do not result in a postsynaptic response, hippocampal circuitry must be very redundant. The fact that the average synaptic current is only about 0.2 nA at peak (1, 11) and the observation that synchronous activity of several dozen synapses is required to reach threshold (12) fit well with this notion of redundancy: no single synapse is very effective in determining a neuron's output, and many must cooperate to produce a spike train. If the estimates for the average number of synapses one neuron contributes to a target neuron are accurate (10, 13–16), the cooperativity must be spread over rather large population of cells. Such a scheme would conform to the requirement that the brain's computational machinery be fault-tolerant.

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