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Feasibility of long-term storage of graded information by the Ca^{2+}/calmodulin-dependent protein kinase molecules of the postsynaptic density

(memory)

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ABSTRACT **The feasibility of long-term information storage by brain type II Ca^{2+}/calmodulin-dependent protein kinase molecules is explored. Recent evidence indicates that this protein has switch-like properties. Equations are derived showing that a single kinase holoenzyme could form a bistable switch having the stability necessary to encode long-term memory, and that a group of kinase molecules, such as that contained within the postsynaptic density, could form a device capable of storing graded information.**

It is generally thought that long-term memory involves changes in the strength of synaptic connections, but the mechanism by which these synaptic "weights" might be stored is completely unknown. One view (1) is that the storage mechanism involves changes in gene expression and is thus dependent on processes in the cell nucleus. An alternative view (2, 3) is that synaptic weights are stored in the cell periphery by devices placed at each of a neuron's synapses. Such devices could not be made of DNA and so would have to be made of unstable molecules such as proteins. In a previous paper (3), a model was presented showing how a stable switch could be constructed out of unstable proteins. This switch could be turned "on" by critical synaptic events and then modify the efficacy of local synaptic transmission. The central component of this switch was postulated to be a group of hypothetical protein kinase molecules having autocatalytic properties. Recent work (4–6) on the brain type II Ca^{2+}/calmodulin-dependent protein kinase, a protein that is thought to be the major protein of forebrain postsynaptic densities (7–9), has shown that this kinase has properties with some important similarities to the hypothetical kinase mentioned above, but with some significant differences. The purpose of this paper is to explore models based specifically on properties of the Ca^{2+}/calmodulin-dependent protein kinase type II. The principal conclusion is that it is theoretically possible for a *single* kinase molecule of this kind to store "on–off" information with the stability necessary to encode long-term memory and for a group of kinase molecules, such as that contained within the postsynaptic density, to form a memory storage organelle capable of storing graded information about synaptic weights.

Fig. 1 summarizes the properties of a Ca^{2+}/calmodulin-dependent protein kinase molecule that enable it to function as a switch. This kinase is a complex holoenzyme composed of about 12 subunits. Four of these are shown in the figure. Each subunit has either two or three principal phosphorylation sites; the holoenzyme thus contains about 30 phosphorylation sites (10, 11). If Ca^{2+} is added, all 30 sites become phosphorylated through an autophosphorylation reaction. Miller and Kennedy (6) found that Ca^{2+} is required only for the addition of the first two to four phosphates (the exact number being uncertain). The addition of these phosphates switches the holoenzyme into a Ca^{2+}-independent "on" state in which both the autophosphorylation of the remaining sites and the phosphorylation of other substrates can proceed even in the absence of Ca^{2+}.

Given these properties, a rise in Ca^{2+} caused by a key neuronal event would be expected to turn "on" kinase molecules. For the kinase to remain "on" after Ca^{2+} returned to baseline, the kinase would have to resist two processes that would tend to reset it. The first of these is dephosphorylation of the holoenzyme by protein phosphatases. The second is protein turnover, which, by replacing phosphorylated protein with newly synthesized unphosphorylated protein, has an effect identical to that of the phosphatase. The resetting effect of the phosphatase itself could be avoided if the rate constant of Ca^{2+}-independent autophosphorylation ($\overline{k_1}$) were significantly greater than the rate constant of phosphatase activity (k_2); under these conditions, phosphates would be added as fast as they are removed and the holoenzyme would therefore stay in the fully phosphorylated "on" state. To avoid the resetting effects of protein turnover, it is necessary that newly synthesized, unphosphorylated, protein be phosphorylated if the protein it replaces was phosphorylated. As previously postulated (3), this could be accomplished if active kinase molecules phosphorylated newly synthesized molecules through an intermolecular reaction. Miller and Kennedy (6) did not detect significant autophosphorylation of one holoenzyme by another, but they did obtain evidence for autophosphorylation of one subunit by another within the same holoenzyme. They suggested that this reaction could enable the holoenzyme to resist the resetting effects of protein turnover, provided that turnover occurred by a subunit exchange process; in this case, a newly synthesized unphosphorylated subunit would become phosphorylated if it is inserted into an active holoenzyme (Fig. 1), but not if it is inserted into an inactive one. Recent work (12) has provided direct evidence that a multisubunit structure undergoes protein turnover by such a subunit exchange process. There are thus feasible mechanisms by which a kinase holoenzyme could resist the resetting effect of both phosphatases and protein turnover.

In the model proposed previously (3), "on–off" information about synaptic efficacy is stored through the interaction of a group of hypothetical kinase molecules, whereas in the model above, "on–off" information is stored through the interaction of subunits within a single Ca^{2+}/calmodulin kinase holoenzyme. Given this storage capacity of single holoenzymes, it becomes possible for a group of ho-

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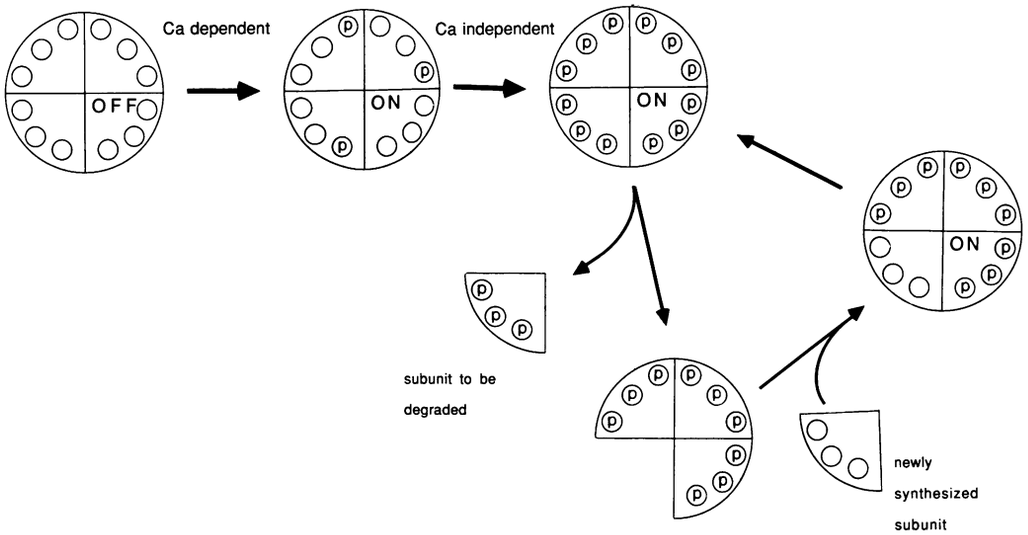

FIG. 1. Ca^{2+} dependent and Ca^{2+} independent steps in the phosphorylation of the Ca^{2+}/calmodulin-dependent protein kinase (4). The kinase has 12 subunits, four of which are shown. The holoenzyme has about 30 phosphorylation sites, 12 of which are shown here. A rise in Ca^{2+} concentration promotes autophosphorylation. If the number of phosphorylated sites reaches a critical number (probably two to four), the autophosphorylation no longer requires Ca^{2+}. Miller and Kennedy (6) suggested that newly synthesized unphosphorylated subunits might exchange with subunits slated for degradation. The newly inserted subunit would become phosphorylated if the holoenzyme had been previously switched "on" by phosphorylation.

loenzymes to store graded information, as illustrated in Fig. 2. Consider a group of four holoenzymes exposed to a brief, saturating pulse of Ca^{2+}. Because the addition of phosphates to the holoenzyme is a probabilistic process, different holoenzymes will contain different numbers of phosphates at the end of the stimulus. If we suppose that N_c, the critical number of phosphates that switch the holoenzyme "on" is 3, then only two of the holoenzymes shown have been switched "on" by the end of the Ca^{2+} pulse. These will become completely phosphorylated after Ca^{2+} is removed, whereas the other two holoenzymes will become dephosphorylated. If, however, the Ca^{2+} stimulus had been much longer, all the holoenzymes would have been switched "on." Thus, the number of holoenzymes switched is graded with the duration of the stimulus.

Fig. 3 shows how the fraction of holoenzymes that is switched "on" varies with the duration of a saturating rise in Ca^{2+}. The derivation of the curves in Fig. 3 *Upper* and the assumptions used are given in the figure legend. Note that what is plotted in the figure is the stimulus duration relative to $t_{0.5}$, the time at which half the holoenzymes have switched "on." The principal conclusion to be drawn from Fig. 3 is that the time range over which switching occurs decreases as N_c is raised. For $N_c = 1$, switching occurs over a very wide time range. This is because there is a large stochastic fluctuation in the time required for an individual phosphorylation. On the other hand, if many phosphorylations are required for switching ($N_c >> 1$), the fluctuations in the timing of individual reactions will tend to cancel and the holoenzymes will switch over a narrow time range. Thus, to encode wide gradations in the duration of a stimulus it is desirable that N_c be as low as possible.

Other functional considerations, however, make it undesirable to make N_c low. These considerations concern switching errors that turn the holoenzyme "on" spontaneously, thereby compromising the integrity of stored information. Such transitions occur in the absence of a stimulus because the rate constant (k_1) of phosphorylation at resting levels of intracellular Ca^{2+} is not 0. If N_c is 1, the rate constant for spontaneous "on" transitions is simply k_1. If N_c is 2 the rate constant is $k_1 f_1$, in which f_1 is the fraction of "off" holoenzymes that has become singly phosphorylated (see Fig. 3 legend for assumptions underlying this and subsequent derivations). The steady-state value of f_1 can be

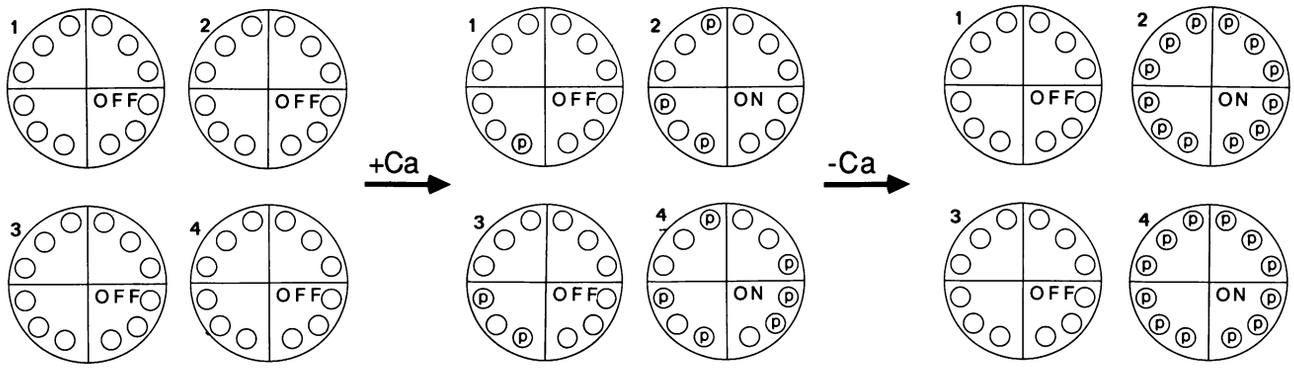

FIG. 2. A scheme by which a group of holoenzymes can store information about gradations in a stimulus. Four holoenzymes start out "off" (*Left*). A brief, saturating pulse of Ca^{2+} leads to partial autophosphorylation of the holoenzymes (*Center*). Because the phosphorylation process is stochastic, different holoenzymes will contain a different number of phosphorylated sites at the end of the stimulus. If the number of phosphorylations required to switch the holoenzyme "on" is three, then holoenzymes 2 and 4 are switched "on" and will subsequently become fully phosphorylated (*Right*), whereas holoenzymes 1 and 3 are "off" and will be dephosphorylated after the Ca^{2+} is removed (*Right*). A much longer pulse of Ca^{2+} would have switched all the holoenzymes "on." Thus, the number of "on" holoenzymes reflects gradations in the stimulus.

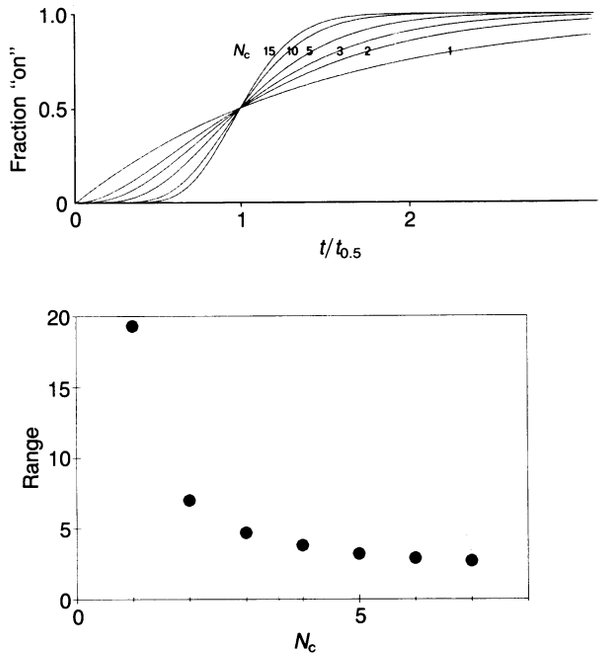

FIG. 3. (*Upper*) Curves showing how the fraction of holoenzymes switched "on" varies with the duration of the stimulus (normalized to the time that causes 50% switching). The curve is shown for different values of N_c, the number of phosphorylations required to switch the holoenzyme "on." In all the derivations given in this paper, it was assumed that the different sites on the holoenzyme become phosphorylated in a fixed sequence. Calculations based on the alternative assumption that all sites are equivalent and phosphorylated independently will be presented elsewhere (13), but they do not lead to substantially different conclusions. The actual situation for the kinase is being actively investigated (14) and will probably be some combination of "sequential" and "independent" mechanisms. The probability of being switched "on" was calculated according to the equation $p_{on} = 1 - p_{off}$, in which the probability of being "off" is $p_{off} = \Sigma_0^{N_c-1} p_n(t)$. Here $p_n(t)$ is the probability of there being n phosphates on the enzyme at time t where $p_n(t) = [(t/\tau)^n/(n!)]e^{-t/\tau}$, as given in standard texts for sequential (see below) irreversible first-order reactions (15). For $\overline{k_1} >> k_2$ the reactions can be accurately modeled as irreversible with $\tau \simeq 1/\overline{k_1}$. If $\overline{k_1}$ were only slightly larger than k_2, more complex equations would have to be used, but the resulting curves would depend similarly on N_c. In calculating the probability of spontaneous "on" transitions it was assumed that there is a steady-state distribution of phosphorylated sites for "off" holoenzymes, that every transition to the "on" state results in a permanent transition (i.e., is irreversible), and that $k_1 << k_2$. Analogous assumptions were made in calculating "off" transitions. These assumptions are not exact, but they are good approximations that are adequate for our present purposes. (*Lower*) Range of encodable stimuli plotted as a function of N_c. Range is defined as the time that the fraction "on" reaches 90% divided by the time at which the fraction is 10%.

closely approximated by $f_1 = k_1/k_2$, in which k_1 is the rate constant for Ca^{2+}-dependent autophosphorylation at resting levels of Ca^{2+} and k_2 is the rate constant of the phosphatase. The general expression for the rate constant of spontaneous "on" transitions for any value of N_c is given by

$$k_1 \cdot (k_1/k_2)^{N_c - 1}. \quad [1]$$

Because k_1 must be smaller than k_2, the rate will fall as N_c is raised. For this reason, switching errors can be reduced by making the switching dependent on multiple phosphorylations. This is so because the individual "error" of spontaneously placing a phosphate on the holoenzyme can usually be corrected by removing the phosphate before another phosphate is added. Thus, only a small fraction of spontaneous phosphorylations leads to switching errors.

It is of interest to estimate an acceptably low rate of switching error for a human memory device and to ask whether this rate is feasible for the Ca^{2+}/calmodulin-dependent kinase. If human memory is to work correctly, the fraction of holoenzymes that switch "on" spontaneously in a human lifetime must be small. Given that the error inherent in the encoding of graded information is of the order of 10% (see below), spontaneous activation of 10% of the holoenzymes during a human lifetime would be tolerable. This translates into a rate constant for switching errors of $3 \cdot 10^{-11}$, a number that can be compared to the rate for the kinase switch calculated according to expression 1 (Table 1). In making these calculations it was necessary to estimate the kinase activity at resting levels of Ca^{2+}. This can be calculated from the known rate (6) at saturating Ca^{2+} ($\overline{k_1}$ = 0.3/sec), the resting level of Ca^{2+}, and the functional dependence of the enzyme activity on Ca^{2+} concentration. This dependence is extremely steep (18) and resembles that of other calmodulin-dependent enzymes (19, 20) that depend cooperatively on the Ca^{2+} concentration, as described by the following equation:

$$k_1 = \overline{k_1} \frac{[Ca^{2+}]^4}{[Ca^{2+}]^4 + (K_d)^4}, \quad [2]$$

in which K_d is the apparent dissociation constant for Ca^{2+} and k_1 is the maximal rate of Ca^{2+}-dependent autophosphorylation, which is assumed equal to the maximal rate of Ca^{2+}-independent autophosphorylation of the switched "on" holoenzyme. If, for example, the resting Ca^{2+} con-

Table 1. Calculations of the rate constant of spontaneous transitions to the "on" state (i.e., the error rate) based on expression 1

	Rate constant, sec^{-1}		
N_c	Case 1	Case 2	Case 3
1	$3.0 \cdot 10^{-5}$	$3.9 \cdot 10^{-6}$	$2.4 \cdot 10^{-7}$
2	$3.0 \cdot 10^{-7}$	$3.0 \cdot 10^{-10}$	$3.0 \cdot 10^{-13}$
3	$3.0 \cdot 10^{-9}$	$2.4 \cdot 10^{-14}$	$3.6 \cdot 10^{-19}$
4	$3.0 \cdot 10^{-11}$	$1.8 \cdot 10^{-18}$	$4.4 \cdot 10^{-25}$

The calculated values should be compared to $3 \cdot 10^{-11}$/sec, the maximum rate that would not significantly compromise the integrity of stored information (see text). The calculations depend on two parameters ($\overline{k_1}$ and $[Ca^{2+}]$) that are known with reasonable accuracy and three parameters (N_c, K_d, and k_2) that can only be estimated to be within a certain range. Cases 1–3 are for different choices of K_d and k_2 within this range; case 1 is least favorable for keeping the error rate low, case 3 is the most favorable, and case 2 is intermediate. $\overline{k_1}$ = rate constant of autophosphorylation in saturating Ca^{2+}. The value is 0.3/sec (6). k_2 = rate constant of dephosphorylation. No experimental information is available about the *in vivo* rate constant. For maintaining the "on" state k_2 must be significantly less than $\overline{k_1}$. The inverse of k_2 is the time constant for dephosphorylation, and this determines the integration time over which neuronal activity leads to switching. For this reason time constants greater than 5 min (rate constant = 0.003/sec) would seem unlikely. For cases 1–3, k_2 = 0.003, 0.05, and 0.2 sec^{-1}, respectively. K_d = apparent dissociation constant for Ca^{2+} defined operationally as the concentration of Ca^{2+} that gives half-maximal stimulation of kinase. This is in the micromolar range, but it is not known with certainty because it depends on the concentration of calmodulin. For the kinase to be switched, Ca^{2+} must rise to K_d or above during neuronal activity; measurements suggest that this rise is at least to 0.5 μM (16). For cases 1–3, K_d = 0.3, 0.5, and 1.0 μM, respectively. $[Ca^{2+}]$ = Ca^{2+} concentration under resting conditions. In hippocampal neurons this is 0.03 μM (16). N_c = number of phosphorylations that causes switching of the kinase to the Ca^{2+}-independent state. This is known to be in the range of 2–4 (6, 17). Calculations are presented for N_c = 1 to illustrate that this value of N_c does not yield an acceptably low error rate.

centration was 0.03 μM and the K_d was 0.5 μM, the kinase activity at resting levels of Ca^{2+} would be about five orders of magnitude lower than at saturation. This illustrates how the cooperative activation of this enzyme by Ca^{2+} enormously reduces the kinase reaction at resting levels of Ca^{2+} and thereby enormously reduces the error rate. Table 1 summarizes calculations of error rates when biochemical parameters are used that are either known or can be estimated to be within a certain range. The calculations show that plausible sets of parameters and values of N_c as low as 2 can lead to an acceptably low rate of spontaneous "on" transitions.

A second type of switching error that could compromise the integrity of stored information is spontaneous transitions of holoenzymes from the "on" to the "off" state. The rate constant for such "forgetting" is

$$k_2 \cdot f_{N_c} = k_2 \cdot (k_2/\overline{k_1})^{30 - N_c}. \quad [3]$$

Because of the high value of the exponent, reverse errors will be extremely infrequent, provided that $\overline{k_1}$ is significantly larger than k_2. Stated qualitatively, the very large number of phosphates that needs to be removed before the holoenzyme is turned "off" makes spontaneous resetting unlikely. These calculations thus demonstrate the feasibility of long-term information storage by the kinase. Furthermore, they provide a plausible explanation for the otherwise puzzling observation that there are so many phosphorylation sites on the holoenzyme, but only a few phosphorylations are needed to switch the holoenzyme "on": the small number of phosphorylations required for switching the kinase "on" ensures a gradedness of the response; the large total number of sites ensures that the probability of spontaneous "off" reactions will be low. Stability requirements also provide an explanation of why "on" kinase molecules must undergo a constant cycle of phosphorylation and dephosphorylation. Such cycles are often termed "futile cycles" because they seem wasteful of energy, but if the cycle were slowed or eliminated by reducing the rate constant for phosphatase (k_2), this would have the undesirable effect of increasing the rate of spontaneous "on" transitions (see expression 1). Thus the "futile cycle" can be viewed as the price that is paid for stability.

The final question concerns the number of holoenzymes that is needed to encode graded information. If there are H holoenzymes in a group, each of which switches with probability, p, the standard deviation of the number switched by identical stimuli is given by the binomial distribution as

$$SD = [H \cdot p \cdot (1 - p)]^{1/2}. \quad [4]$$

The limitation this variability places on the accuracy of encoded information is shown in Fig. 4 for two group sizes: H = 10 and 100. It can be seen that the group of 100 holoenzymes is capable of encoding information to an accuracy of about 10%, but that the smaller group is not. There is no firm number available for the number of holoenzymes in the postsynaptic density. If holoenzyme [approximately 20 nm in diameter (10)] were packed into a two-dimensional array with a center-to-center spacing of 30 nm, approximately 80 holoenzymes could fit into the postsynaptic density [diameter = 300 nm; thickness = 50 nm (21)]. Biochemical estimates (22) suggest that the number may be even higher. Thus the number of holoenzymes in the postsynaptic density suggests that it can store graded information to an accuracy of about 10%.

The principal conclusion of this paper is that there is no theoretical obstacle to the proposal that the postsynaptic density is a memory storage device capable of storing graded information in a stable manner. The mechanism of storage is based on the ability of the Ca^{2+}/calmodulin-dependent protein kinase holoenzymes that are present in the postsynaptic density to store binary information. However, the storage of graded information should be viewed as analog rather than digital because the encoding depends not on which holoenzymes are switched but only on the number that are switched. At this point the evidence supporting this view of memory storage is entirely circumstantial. Recent evidence suggests that associative learning may depend on critical events leading to Ca^{2+} influx through *N*-methyl-D-aspartate receptors on postsynaptic cells (23–27). The position of the postsynaptic density and the properties of the kinase within it would seem well suited for detecting and remembering such events.

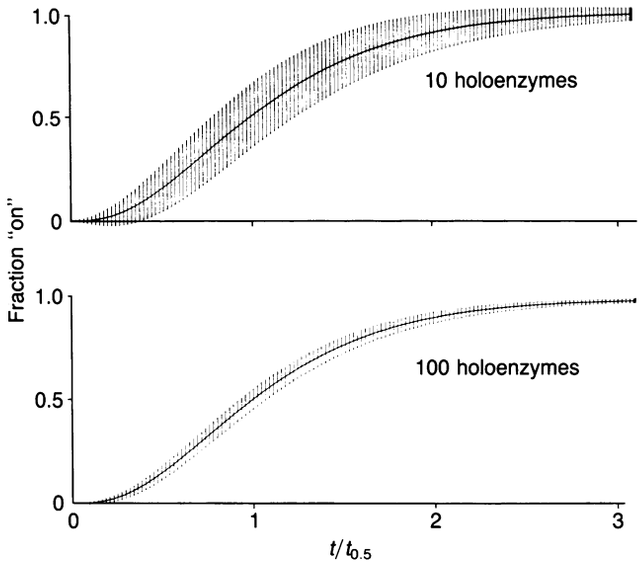

FIG. 4. Accuracy of encoded information depends on the number of holoenzymes (H). The plots show the standard deviation of the fraction of holoenzymes switched "on" by stimuli of time t. (*Upper*) Group of 10 holoenzymes; (*Lower*) group of 100. If the encoding of stimulus duration were exact (standard deviation equal to 0), a unique stimulus duration could be inferred from knowing the fraction of holoenzymes switched "on." Because the standard deviations are not 0, the duration of the stimulus can be encoded with only limited accuracy. The curves were calculated as described in the Fig. 3 legend for N_c = 3. The standard deviations were computed according to Eq. 4.

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