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Temporal summation in rat prefrontal pyramidal cells. Differential effects of pre- and postsynaptic neurochemical manipulations

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The influence of both reserpine-induced depletion of catecolamines and chlorpromazine-induced blockade of dopamine receptors on integrative properties of rats prefrontal pyramidal cells was studied by evocating threshold direct cortical responses with different trains of electrical pulses. Catecholamine depletion results in increased pulse train stimulating currents for eliciting threshold cortical responses, whereas chlorpromazine blockade of dopamine receptors results in higher time constants characterizing postsynaptic temporal summation. It is suggested that the leaky-integrator neuron model may be a discriminating paradigm for detecting electrophysiologically the pre- or postsynaptic level of occurrence of some synaptic disorders.

The leaky-integrator neuron model provides a very simplified way for analyzing many of the relevant dynamic aspects of input–output relationship of synapses, as can be noted from both theoretical[4,13,20,26,28,31] and experimental[3,7,9,13,23] approaches. Here we used this formal model as a paradigm for detecting the pre- or postsynaptic level of ocurrence of a neurochemical manipulation we imposed in the rat prefrontal cortex.

From a general point of view, stimulation of presynaptic fibers with evenly spaced electrical pulses provides discrete release of neurotransmitter; these neurotransmitter 'packets' generate transient transmembrane currents at the postsynaptic membrane which are temporarily added, the cumulated electrical charge being estimated by the postsynaptic potential magnitude. In excitatory synapses, appropriate current intensity for a determined train stimulus duration provides the required excitatory postsynaptic potential (EPSP) to trigger the neuronal firing. These are, essentially, the transformation properties of nerve cells which are described by the leaky-integrator neuron model. In its more elementary version, the leaky-integrator can be considered as formed by a resistor-shunting capacitor.

The strength–duration data of pulse train electrical stimuli required for eliciting neuronal firing theoretically fit with an exponential function, $i(t) = Q/KT\,[1 - \exp(-t/T)]$, where i is the effective train current intensity, Q is the cumulated postsynaptic electrical charge required for neuronal firing, T is the time constant and t is the train duration. Strictly, the 'biological' leaky-integrator is constituted by the postsynaptic membrane, which integrates the chemically gated transient transmembrane currents. However, the postsynaptic transmembrane current could be taken as linear with the stimulating train current, on the basis of neurotransmitter substances being produced at a rate proportional to the exciting stimulus[5]. In the formalism stated above, K is the appropriate factor transforming the stimulating currents i into the postsynaptic transmembrane currents i', so that $i' = Ki$. Thus, K is a factor depending mainly on the efficiency in transforming electrical stimuli into stimulated-release of neurotransmitter at the preterminal endings. It can be noted, therefore, that both K and T parameters are respectively characterizing presynaptic as well as postsynaptic dynamic aspects of neuron excitation.

The experiments were carried out on 13 young Wistar rats (38–43 days old), slightly anesthetized with 100 mg/kg i.p. of α-chloralose, according to procedures described elsewhere[11]. A single dose of 3 mg/kg of D-tubocurarine was injected as a muscle re-

laxant, and adequate ventilation was maintained by means of a respirator pump. After exposure of the frontal lobe of one cerebral hemisphere, bipolar stimulation of the cortical surface was carried out by means of two silver ball electrodes of 0.5 mm each. Direct cortical evoked responses were unipolarly recorded from the prefrontal cortex, so that stimulating (caudal) and recording (rostral) electrodes formed a 1.5 mm side length triangular array located between Groot's stereotaxic coordinates, A: 10.0-11.5 and L: 1.0-2.5, in millimeters[21]. Cortical stimulation consisted of trains of rectangular pulses of 0.1 ms duration per pulse and 1000 Hz frequency, delivered from a Tektronix Stimulator 161 equipped with a Grass SIU 5 Stimulus Isolation Unit. Mean train current intensity and train duration were adjusted so that threshold direct cortical responses (DCR) of 0.5 mV amplitude were evoked. The stimulating current was conventionally measured by inserting a 4.7 kohm resistor in the circuit. Recordings were amplified by a Tektronix 122 preamplifier (8–10000 Hz bandwidth), displayed on a Tektronix 502-A oscilloscope and digitized at a 1000/s rate by an A/D converter interfaced to a Rockwell AIM 65 microcomputer. They were also stored on magnetic tape for retrieval.

Seven rats were submitted to the experimental conditions prior and 15 min after an i.v. injection of chlorpromazine 5 mg/kg. This drug acts as an antagonist of dopamine mainly at the D2 type receptors[29], interfering therefore with postsynaptic binding of the neurotransmitter. Another 6 rats were injected with reserpine 2.5 mg/kg i.p. 24 h before the beginning of the electrophysiological experiment in order to deplete catecholamines[30]. The parameters K and the time constant T were respectively taken, according the model, as indicators of presynaptic and postsynaptic effects of the drug administered.

Computation of the function i(t) allowed us to obtain the expression ln [d(1/i)/dt] as a function of t in all groups of rats studied. Hereafter, this latter will be referred to as function F(t) (see Appendix). Since the function F(t) is almost linear ($r > 0.85$), the train-stimulating current i is therefore an exponential function of time, in accordance with the prediction of the leaky-integrator neuron model (Fig. 1). The function F(t) actually permits a calculation of the time constant of pyramidal cells from the slope of the regression line; in addition, it permits evaluation of the K factor through determining the quotient Q/K from the Y intercept, since Q remains constant.

The results showed that animals receiving reserpine did present smaller K factors compared to controls ($P < 0.005$), as can be inferred from the larger Q/K of the former group (Fig. 1). This resulted in increased train stimulating currents for the catecholamine-depleted rats, as can be noted from the smaller K factor (about one half) obtained in these animals. Besides, rats receiving chlorpromazine showed significantly higher time constants than control ($P < 0.001$); in addition, they also showed smaller K factors than normal rats but the difference was not statistically significant (Fig. 1).

The significance of the leaky-integrator neuron model as a paradigm for discriminating the level of occurrence of synaptic disorders in the cerebral cortex requires some considerations: (1) one must know which elements were stimulated, if an interpretation at a cellular level is needed. This question has been largely emphasized by Ranck[27]. With respect to this, it is known that weak electrical stimulation of the cerebral cortex surface in order to evoke DCRs involves excitation of superficial presynaptic endings forming axodendritic synapses with apical dendrites[24,25], the DCRs being the extracellular recordings of averaged EPSPs of the neighboring population of neurons[22,24]. As has been pointed out[24], weak cortical stimulation providing surface negative DCRs implicates the excitation mainly of excitatory axodendritic synapses; therefore, it can be assumed that the results obtained are characterizing the neuronal integration of EPSPs. In the rat prefrontal cortex a major proportion of synapses are constituted by dopaminergic axon terminals of the mesencephalic ventromedial tegmentum which ends on cortical layers II to VI[1,15]. However, the DCRs recorded are certainly also contributed by excitation of noradrenergic and glutamatergic projections existing in this cortical area[8]; (2) strength–duration data of train stimuli must fit with an exponential function; otherwise, the analysis would be limited only to an empirical relation. In this respect, the high X–Y linear correlation ($r > 0.85$) of the regression lines in all groups, obtained from plotting the function F(t), implicates that the strength–duration curves for threshold cortical activation are exponential functions. This is in agreement with data reported by Gallistel[10] indicat-

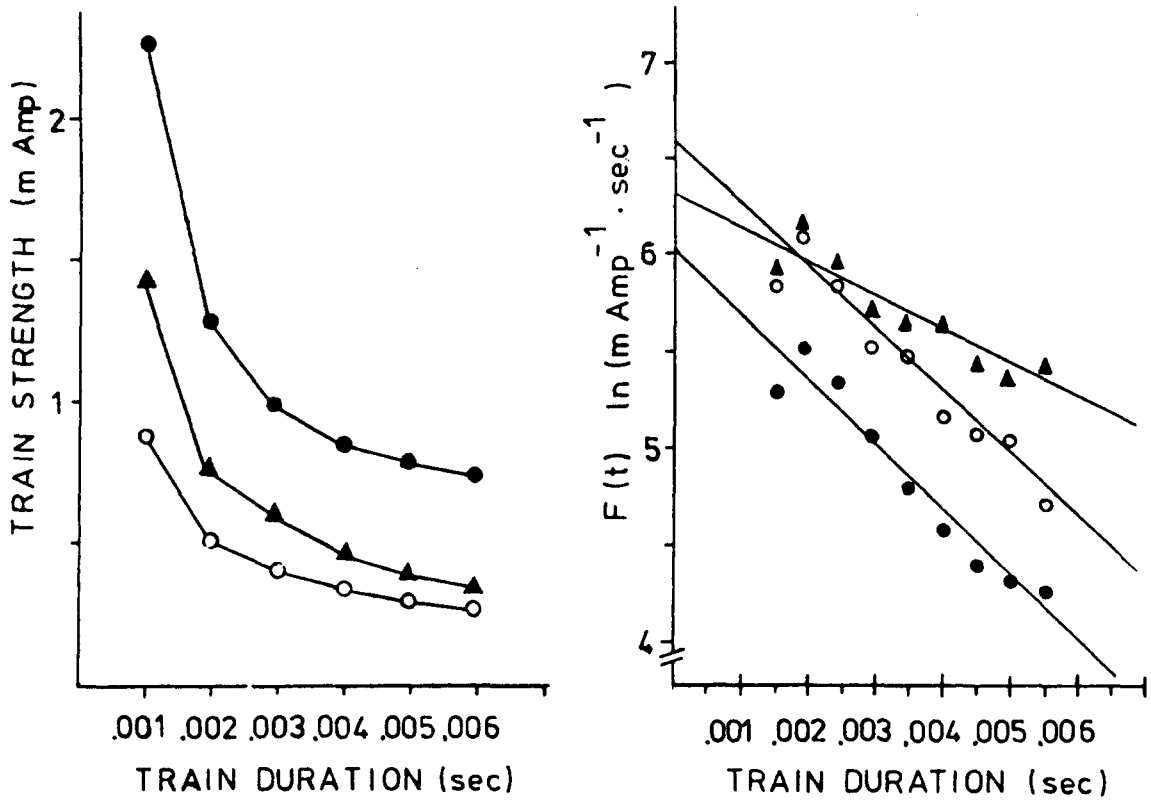

Fig. 1. Left graph: strength–duration curves of pulse train electrical stimuli for eliciting threshold direct cortical responses in the rat prefrontal cortex. Each point is the arithmetic mean of the required currents in normal (open circles), reserpine-injected (closed circles) and chlorpromazine-injected rats (closed triangles). Right graph: least-squares regression lines fitting the (F,t) plots. Open circles: normal rats (the regression line obeys the equation $F(t) = -325t + 6.61$; $r = 0.89$; T = 3.07 ms; Q/K = 1.33 μCoulomb). Closed circles: reserpine-injected rats (the regression line obeys the equation $F(t) = -349t + 6.02$; $r = 0.94$; T = 2.86 ms; Q/K = 2.42*μCoulomb). Closed triangles: chlorpromazine-injected rats (the regression line obeys the equation $F(t) = -171t + 6.29$; $r = 0.85$; T = 5.82**ms; Q/K = 1.83 μCoulomb). * $P < 0.005$, ** $P < 0.001$, respect to normal rats (Student's t-test).

ing that the strength–duration plots obtained by pulse train stimulation of the hypothalamus closely fit an exponential function. Moreover, Libet[14] has reported that strength–duration curves of cortical train stimuli for eliciting both objective motor and subjective sensory threshold responses in man appear to be analogous to the strength–duration curve for threshold excitation of nerve fibers. As is known, this latter is an exponential relation constituting the Blair's equation[2].

In the light of these considerations, the increased stimulating electrical currents required by catecholamine-depleted rats (about 2-fold, compared to normal rats) for eliciting threshold DCRs would be in agreement with the prediction of the leaky-integrator neuron model, since prefrontal endings of depleted rats obviously need a larger stimulus strength for releasing the required amount of neurotransmitter than normal rats. In other words, the K factor of catecholamine-depleted rats is nearly half that observed in normal animals. Likewise, the unchanged time constant values of pyramidal cells after reserpine-induced catecholamine depletion was also expected since this drug acts solely at the presynaptic level.

On the other hand, the increased time constants observed in pyramidal cells of prefrontal cortex of rats injected with chlorpromazine must be produced as a cause of the larger postsynaptic membrane resistance during chemical gating in this group compared to normal animals, on account of dopamine D2 receptor blockade. The values reported here actually correspond to the average activity of various particular postsynaptic elements activated because field evoked potentials were used as an indicator of threshold excitation. These values are in the range of the 1–10 ms neuronal time constant reported by oth-

ers[16,19,27]. As has been pointed out, the time constant characterizing neuronal temporal summation might also depend on the mean life of the neurotransmitter molecules after receptor binding[6]. This observation suggests that monoamine oxidase inhibitors could modify the time constant of neurons receiving monoaminergic terminals, by inducing longer opening of postsynaptic channels. Nevertheless, this point requires further experimental confirmation.

In addition, the chlorpromazine injected rats also showed smaller K factors than normal rats. That means chlorpromazine also interacts at presynaptic levels in the rat prefrontal cortex, notwithstanding that this effect was statistically insignificant. With respect to this, the existence of dopamine D3 autoreceptors has been reported situated in the preterminal endings[12,32]. The presynaptic D3 receptor can be blocked by neuroleptics such as haloperidol or chlorpromazine, resulting in both an increase or a decrease of the stimulated release of dopamine according to neuroleptic concentration[18,29]. Thus, assuming that chlorpromazine blockade of D3 receptors may be producing a lower release of dopamine, the larger stimulating currents required by rats receiving this drug is not surprising. It is likely that changes in stimulating currents may depend on some arbitrary experimental choices; for instance, the distance between stimulating electrodes, the impedance of the electrode saline junction or the stimulating electrode surface. Therefore, measurements of the parameter K would have a reasonably significant meaning only when these experimental choices are carefully characterized and standardized. Unlike currents or voltage measurements, the time constant appears to be an intrinsic independent parameter for quantitative characterization of transformation properties of synapses.

Finally, it seems important to note that train stimulation implicates some nonlinearity in the model, since both facilitation and postactivity potentiation has been reported during repeated presynaptic stimulation[5]. Other sources of nonlinearities inherent to repetitive stimulation could be accommodation, threshold change, habituation, etc. These were neglected in this study since the equations would became too unwieldy. The nonlinear behavior may perhaps explain the bad fit of the points corresponding to the shortest train stimulus duration (0.001 s), as can be seen in all regression lines of Fig. 1. If this point is not computed, a considerable improvement in the correlation coefficients is obtained in all groups tested ($r > 0.93$).

Al these data are indicative that superficial stimulation of the rat prefrontal cortex with pulse train electrical stimuli constitutes a paradigm closely resembling the leaky-integrator neuron model, suggesting that this type of approach may be useful for detecting the level of occurrence of gross synaptic disorders produced in experimental models of metabolic diseases.

APPENDIX

The strength–duration function, i(t), of train pulse currents required for 'firing' the leaky-integrator neuron model, that is a resistor-shunting capacitor, is given by:

$$i(t) = \frac{Q}{K\,T\,(1 - e^{-t/T})} .$$

We shall now describe a way of obtaining T and Q/K from any given i and t experimental data.
First, we express 1/i as function of t:

$$\frac{1}{i} = \frac{K\,T}{Q} - \frac{K\,T}{Q}\, e^{-t/T} \qquad (1)$$

Eqn. 1 cannot be simply linearized. However, the slope of the curve 1/i (t) can be computed or calculated by graphic methods for many particular time intervals, permitting the plotting of d(1/i)/dt as function of t. Then, by deriving Eqn. 1 in respect of t, it remains:

$$d(1/i)/dt = \frac{K}{Q}\, e^{-t/T} \qquad (2)$$

The expression given in Eqn. 2 can be linearized by taking the logarithm in both members of Eqn. 2:

$$\ln [d(1/i)/dt] = -\frac{1}{T}\, t + \ln \frac{K}{Q}$$

This linear relation is called as function F(t). T can be calculated as minus the reciprocal of the slope and Q/K as the reciprocal of the antilogarithm of the Y intercept.

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