Introduction

A recent experimental study on the firing patterns of midbrain dopamine cells in freely-moving, unanaesthetized rats [5] revealed that some neurons fired in an irregular fashion, but that a sizeable minority of the dopamine neurons in unanaesthetized animals fired in an extremely regular clock-like activity pattern. Tepper et al [10] also observed regular rhythmic firing as well as irregular firing in anaesthetized rats, and noted transitions between firing modes following manipulation of afferent inputs. In a slice preparation, dopamine neurons generally fire in a rhythmic, pacemaker-like fashion. The slice preparation is lacking most afferents, including the glutamatergic afferents often associated with burst firing in these neurons. The calcium activated SK potassium channel is responsible for the afterhyperpolarizing potential that follows an action potential during pacemaker firing [7]. Apamin blocks the SK channel, and the bath application of apamin to cells firing in a pacemaker-like fashion in vitro can convert the firing pattern to irregular firing (see Fig. 1), more than doubling the coefficient of variation (CV) from about 0.09 to 0.26.

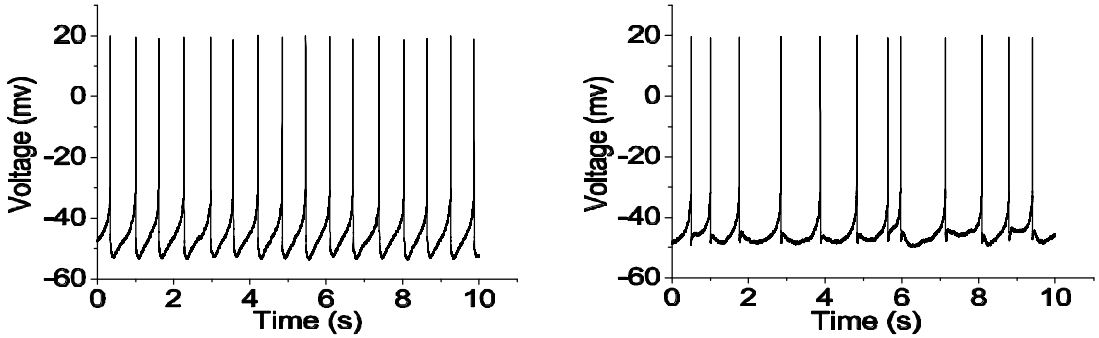

Figure 1. Effect of bath applied apamin on pacemaker firing in dopamine neurons in vitro. The motivation for this study was provided by two sets of experiments on dopamine neurons. Unpublished data provided by Lucy Clerkin in Paul Shepard's lab at the University of Maryland Psychiatric Research Center.

We hypothesized that spontaneous inhibitory events in the slice were more effective in disturbing the regularity of the firing pattern because of the way in which the application of apamin changes the slope of the membrane potential waveform between spikes (see results).

The only published phase resetting data [6] on dopamine neurons with which we are familiar used a slice preparation in which also contained the subthalamic nucleus (STN). Subthreshold and suprathreshold excitatory perturbations were induced by stimulating the STN. Although only a few examples were shown in the figures of that paper, a subthreshold excitatory perturbation advanced the timing of the subsequent spike, and the rhythm with the next ISI corresponding to the intrinsic period. On the other hand, a suprathreshold perturbation appeared to cause an ectopic spike and the next spike appear to occur about when it would have been expected in the absence of the ectopic spike. The experimentalists concluded that the pacemaking mechanisms were spatially separated from the spike generating mechanisms snce the spike did not appear to reset the phase. We examined the qualitative difference between phase resetting due to subthreshold and suprathreshold perturbations in a simple model (see results).

Results

In vitro, spontaneous inhibitory postsynaptic currents (sIPSCs) corresponding to activation of $GABA_A$ receptors comprise virtually all of the spontaneous synaptic activity [4] , and they generally occur without any organized pattern at a mean frequency of 7.52 ±1.22 Hz [1]. It is reasonable to assume that these randomly timed events induce phase resetting (a shortening or lengthening of the cycle period in response to a transient perturbation). For Type I excitability, the amount of phase resetting is inversely proportional to the total ionic membrane current, as well as to the slope of the membrane potential waveform [8]. The subthreshold variations in membrane potential appear much smaller in the presence of apamin, leading to a smaller value of the slope and a larger amount of phase resetting due to a constant IPSC. Simulations of type I oscillators that show that the CV increases markedly in the presence of constant levels of random background synaptic activation when the membrane potential waveform is "flattened out" near threshold for a repetitively firing pacemaker neuron, hence this is one possible mechanism by which apamin could cause the firing pattern to become irregular (see Figure 2).

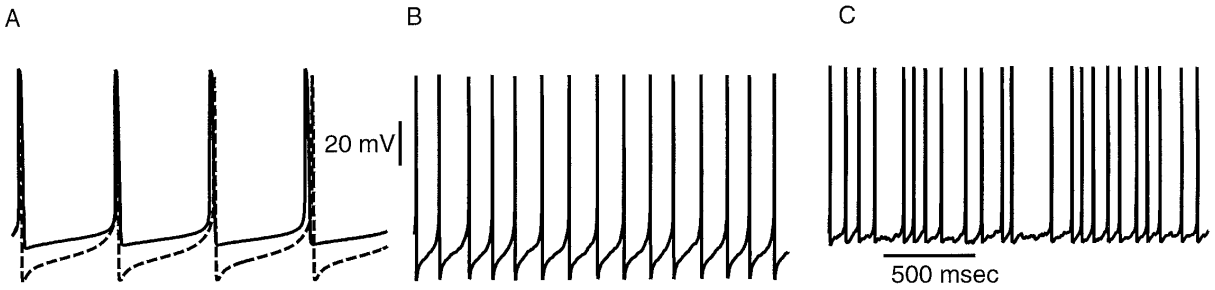

Figure 2. Effect of the waveform on the amount of irregularity introduced by background synaptic noise. A) The dashed waveform corresponds to the unperturbed, free-running pacemaker firing that is subjected to noise in B). The solid waveform corresponds to the unperturbed pacemaker firing corresponding to the unperturbed, free-running pacemaker firing that is subjected to noise in C). The time scale bar is 500 msec for A and 5000 msec for B and C.

We used a simple model, one compartment model previously used by Plant [9]) in a Type I spiking regime to illustrate the effect of the waveform on the susceptibility to noise. In Type I excitability, spiking arises via a saddle node bifurcation[3], and the frequency can be adjusted to be arbitrarily slow by adjusting the amount of injected current. We used two sets of parameters. In Fig 2A, the pacemaker oscillations produced at these two parameter sets are shown. In Fig. 2B,

The waveform corresponding to the dashed lines in Fig. 2A was subjected to a series of inhibitory postsynaptic potentials (IPSP) whose interevent times were generated by a simulated Poisson point process with a mean interevent time of 50 msec. The IPSP was generated by delivering a 5 msec pulse to a damped second order linear oscillator, and the response of the oscillator was used as a transient perturbation in synaptic conductance [2]. Just as in Fig. 1A, the pacemaker firing seems largely undisturbed by the barrage of synaptic input. In order to simulate the irregular firing in Fig. 1B, we assumed that apamin doubled the intrinsic firing rate, therefore we doubled the mean interevent time in Fig 2C to account for the effect of frequency. At twice the frequency, on average half as many IPSPs will be received per cycle. In order to show the effect of increasing the frequency, Fig. 2C is shown on a compressed time scale. Even though on

average half as many synaptic inputs are received per cycle in Fig. 2C compared to Fig. 2B, their effect is much greater causing the firing to appear more irregular and the CV to increase.

We next examined the effect of excitatory postsynaptic potentials on a third set of model parameter, still in the Type I range but set to increase the influence of calcium influx during the subthreshold portion of the oscillation on the calcium-activated current. Just as in Kang and Futami [6], a subthreshold perturbation advanced the phase (Fig. 3A) whereas a suprathreshold perturbation (Fig. 3B) applied at the exact same phase appeared to induce an ectopic spike without resetting the phase. However, a closer examination indicates that the spike after the "ectopic" spike in Fig. 3B actually occurs later than if no perturbation had been applied, and a close examination of Fig 3 in [6] produces similar results. In the phase space of the model (not shown) the trajectory of a supratheshold perturbation is quite different than that of a subthreshold perturbation. No separation in the model between spike generating and pacemaking methods is required.

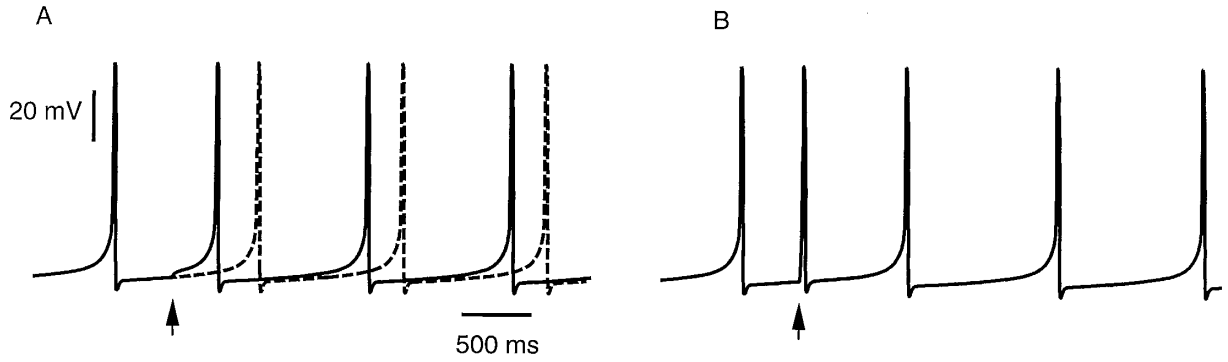

Figure 3. Phase resetting induced by subthreshold versus suprathreshold perturbations. A) A subthreshold pertutrbation (arrow) caused an advance (unperturbed waveform given by the dashed lines). B. A suprathreshold perturbation appears to induce an ectopic action potential without resetting the phase (but see text).

Conclusions

A biophysical mechanism by which modulation of the firing pattern in spontaneously repetitively firing neurons is proposed that can generalized to other neurons and other modulators, because the only relevant parameter is the slope of the potential waveform between action potentials. Furthermore, it was shown that a spatial separation between pacemaking and spike generating mechanisms is not required to produce a qualitatively different phase resetting in response to subthreshold versus suprathreshold perturbations.

References

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