Dopamine Neuron Responses Depend Exponentially on Pacemaker Interval

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Abstract

Midbrain dopamine neuron activity results from the integration of the responses to metabotropic and ionotropic receptors with the postsynaptic excitability of these intrinsic pacemakers. Interestingly, intrinsic pacemaker rate varies greatly between individual dopamine neurons and is subject to short- and long-term regulation. Here responses of substantia nigra dopamine neurons to defined dynamic clamp stimuli were measured to quantify the impact of cell-to-cell variation in intrinsic pacemaker rate. Then this approach was repeated in single dopamine neurons in which pacemaker rate was altered by activation of muscarinic receptors or current injection. These experiments revealed a dramatic exponential dependence on pacemaker interval for the responses to voltage-gated A-type $K^+$ channels, voltage-independent cation channels and ionotropic synapses. Likewise, responses to native metabotropic (GABAb and mGluR1) inhibitory synapses depended steeply on pacemaker interval. These results show that observed variations in dopamine neuron pacemaker rate are functionally significant because they produce a >10-fold difference in responses to diverse stimuli. Both the magnitude and the mathematical form of the relationship between pacemaker interval and responses were not previously anticipated.

Keywords: dynamic clamp, brain slice, Kv4.3 potassium channel, antipsychotic drug, substantia nigra
Introduction

Dopamine (DA) neuron activity in vivo displays a variety of patterns including bursts and regular rhythmic activity (Wilson et al., 1977; Grace and Bunney, 1984a,b; Hyland et al., 2002). These patterns arise from the interaction of ionotropic and metabotropic receptor responses with the intrinsic excitability of these neurons. Unlike most neurons in the brain, DA neurons spontaneously produce repetitive regular activity after isolation or reduction of synaptic input (i.e., by generating brain slices) (Kita et al., 1986; Hainsworth et al., 1991). In fact, this intrinsic pacemaker activity is highly variable between individual DA neurons and subject to short- and long-term regulation (Dai and Tepper, 1998; Franz et al., 2000; Liss et al., 2001; Yang et al., 2001; Hahn et al., 2003, 2006). Because DA neurons don’t control timing (i.e., the role of many pacemakers), their pacemaker properties may influence computation. Specifically, there must be some effect of the variations in intrinsic excitability that produce pacemaker rate differences (Liss et al., 2001; Hahn et al., 2003, 2006) on the efficacy of synapses, modulators and drugs (e.g., near the maximal firing rate, responses will be reduced). Yet, the impact of variation in intrinsic pacemaker activity has not been measured. Furthermore, it is unclear whether all DA neuron responses share the same dependence on pacemaker rate.

Determining the relationship between the DA neuron responses and their varied intrinsic pacemaker rates is technically challenging with standard approaches. First, synapses are usually studied in isolation by preventing pacemaker activity (i.e., in hyperpolarized cells), while the intrinsic pacemaker mechanism is often studied in the absence of synaptic activity. Second, because each DA neuron is regulated by a large
variety of synapses, it is difficult to stimulate an identical input on individual DA neurons with varied intrinsic properties. These difficulties can be bypassed with the dynamic clamp, a method that allows the investigator to acutely and specifically add virtual channels to a single neuron (Prinz et al., 2004). The dynamic clamp has already been applied to study Kv4.3 channels in cultured DA neurons and episodically activated synaptic channels in other neurons (Desai and Walcott, 2006; Hahn et al., 2006; Kullmann and Horn, 2006; Fernandez and White, 2008). Thus, the dynamic clamp could be used to directly quantify how variations in DA neuron pacemaker rate found in substantia nigra brain slices affect the response to experimentally defined stimuli.

This study begins by using virtual Kv4.3 channels as a defined stimulus because native Kv4.3 channels mediate acute and long-term modulation of DA neuron excitability induced by GDNF and D2 autoreceptors, respectively (Yang et al., 2001; Hahn et al., 2003, 2006). After determining that pacemaker diversity between DA neurons has a dramatic impact, the effect of pacemaker rate is shown to apply to individual DA neurons in which activity is altered by current injection or activation of native muscarinic receptors. This steep exponential relationship is also conserved for responses to virtual voltage-independent cation channels, virtual ionotropic synapses and native metabotropic synapses. Hence, known variations in pacemaker rate are functionally significant because they alter DA neuron responses to a wide variety of stimuli by >10-fold.
Materials and Methods

Brain slices. Reagents were from Sigma-Aldrich, if not stated otherwise. All experiments were conducted in accordance with protocols approved by the University of Pittsburgh Institutional Animal Care and Use Committee. Sprague Dawley rats (postnatal days 14-21, Hilltop Labs) were anesthetized with isoflurane and decapitated. The brain was removed and placed into ice-cold, 95% O₂ and 5% CO₂-saturated, sucrose-based artificial cerebrospinal fluid (s-aCSF, in mM: 87 NaCl, 75 sucrose, 2.5 KCl, 25 NaHCO₃, 1.25 NaH₂PO₄, 0.5 CaCl₂, 7.0 MgSO₄, 25 glucose, 0.15 ascorbic acid, 1 kynurenic acid, pH 7.4). Coronal midbrain slices (250 μm) were cut on a vibratome (Vibratome 3000, The Vibratome Company). The slices were incubated in s-aCSF at room temperature for at least one hour before the experiments.

Patch clamping. Substantia nigra pars compacta DA neurons were identified based on location, morphology and characteristic electrophysiological properties (Grace and Bunney, 1983; Lacey et al., 1989; Dai and Tepper, 1998; Neuhoff et al., 2002; Paxinos and Watson, 2005). Whole-cell recordings were performed at 30-32°C, using an AM Systems 2400 amplifier. This patch clamp amplifier is set up for fast true current clamp recording, which is required for dynamic clamp. The resistance of patch-clamp electrodes was 2-4 MΩ. The pipette solution (in mM: 120 potassium gluconate, 20 KCl, 10 HEPES, 2 MgCl₂, 0.1 EGTA, 1.2 ATP, pH 7.3) was chosen so that pacemaker activity recorded initially in the on-cell configuration (Perkins, 2006) was not perturbed following breaking into the whole-cell configuration. Over the course of experiments (up to 30 minutes after changing to whole-cell configuration), no differences in mean ISI and its coefficient of variation were detected (n=8; data not shown). Oxygenated standard
aCSF (in mM: 124 NaCl, 4 KCl, 25.7 NaHCO₃, 1.25 NaH₂PO₄, 2.45 CaCl₂, 1.2 MgSO₄, 11 Glucose, 0.15 ascorbic acid, pH 7.4) was superfused over the slice at 1 - 2 ml/min. Inclusion of blockers of AMPA, NMDA and GABA_A receptors did not affect pacemaker activity, showing that background synaptic activity was not significant (n=11; data not shown).

**Dynamic clamp.** The dynamic clamp setup and the virtual DA neuron Kv4.3 A-type K⁺ conductance, the non-specific cation conductance and implementation of virtual synaptic conductances have been described previously (Kullmann et al., 2004; Hahn et al., 2006; Kullmann and Horn, 2006). In brief, the equation for A-type K conductance was \( I_A(V,t) = g_A m(V,t) h(V,t) (V - E_{rev}) \), with \( E_{rev} = -84 \text{ mV} \), \( dm(V,t)/dt = [m_\infty(V) - m(V,t)]/\tau_m(V) \), \( m_\infty(V) = 1 / [1 + \exp(-V + 24.8) / 13.9] \), \( \tau_m(V) = 2 - (1.6 / [1 + \exp(-(V + 20) / 15])] \), \( dh(V,t)/dt = [h_\infty(V) - h(V,t)]/\tau_h(V) \), \( h_\infty(V) = 1 / [1 + \exp(-(V + 78.7) / 9.2)] \), and \( \tau_h(V) = 28 - (9.4 / [1 + \exp(-(V - 2) / 16])] \). The equations for the synaptic conductance were \( I_{syn}(t) = g_{syn}(t) (V - E_{rev}) \) and \( g_{syn}(t) = k [\exp(-t/\tau_{rise}) - \exp(-t/\tau_{fall})] \), with \( \tau_{rise} = 1 \text{ ms} \), \( \tau_{rise} = 5 \text{ ms} \) and \( E_{rev} = 0 \text{ mV} \) for excitation and -65 mV for inhibition.

**Stimulation of native synapses.** Field stimulation of the substantia nigra pars reticulata was induced with square-wave pulses (10-60 V, 100 µs) through a bipolar stainless-steel stimulating electrode placed 200-1000 µm away from the recorded neuron (Mereu et al., 1991). The stimulator was triggered by the same computer generated random 5 Hz protocol used for dynamic clamp application of virtual synaptic conductances.

**Drug application.** Where indicated, ionotrophic receptor inhibitors were superfused over the slice: 50 µM picrotoxin (a GABA_A receptor antagonist), 50 µM D-AP5 (an NMDA-receptor antagonist) and 10 µM CNQX (an AMPA/kainate receptor antagonist). To block
metabotropic inhibitory receptors, 30 µM CGP35348 (a GABAb receptor antagonist) and 100 µM 1-Aminoindan-1,5-dicarboxylic acid (AIDA; an mGluR1 receptor antagonist) were superfused over the slice. Muscarine was added by superfusion at a final concentration of 1 µM.

Data analysis. Data are displayed as mean with bars indicating standard error of the mean (SEM). Linear and nonlinear regression fits, along with 95% confidence intervals (displayed as dashed lines), were calculated with Graphpad Prism. Mean baseline and altered steady state interspike intervals (ISIs) were calculated from at least 10 values. The initial change in ISI (ΔISIi) equaled the difference between the first ISI after changing firing (e.g. after addition of A-conductance) and baseline ISI (ISIb), while steady state ΔISI was calculated as the absolute difference of ISIb and new steady state ISI (e.g. from the last 20 seconds of the period after ISI is altered).
Results

The relationship between pacemaker interval and the response to A-type K⁺ channels

Spontaneous pacemaker activity of substantia nigra pars compacta DA neurons was recorded in rat brain slices. As described in the Materials and Methods, these measurements were not affected by the recording configuration or background synaptic activity. We began our analysis by using the dynamic clamp to add 100 nS of Kv4.3 A-type K⁺ channel conductance (gKₐ) to DA neurons with diverse intrinsic pacemaker rates. Kv4.3 channels were chosen because both their gating and expression are controlled in DA neurons and this channel has already been implemented in the dynamic clamp (Yang et al., 2001; Hahn et al., 2006). Thus, the dynamic clamp could be used to quantify whether the impact of such changes in Kv4.3 activity would be significantly affected by the known variation in pacemaker rate found among individual DA neurons.

Addition of virtual A-type channels elicited an initial increase in the interspike interval (ISI), which was then followed by a period of adaptation before a new steady state pacemaker rate was established (Fig. 1A,B). However, the size of the response depended dramatically on baseline pacemaker interval (Fig. 1A,B; note the varying y axis scales in B). Linear and semi-log plots show that both the initial and steady state changes in ISI (ΔISIᵢ and ΔISI) increased exponentially with baseline ISI (ISIᵢ) (Fig. 1C-F). The similarity in the relationships for the initial and sustained changes in firing implies that the effect of pacemaker interval on the response to A-type channels is independent of adaptation. Furthermore, the fit over a wide range of intervals implies that this relationship does not reflect a simple saturation effect. Most importantly, the
diversity of pacemaker rates among individual DA neurons is associated with dramatic variation in the response to a change in A-type channel activity.

The pacemaker interval effect is evident in individual DA neurons

To determine whether the pacemaker interval effect is an intrinsic property of individual DA neurons, the effect of adding virtual A-type channels was tested under control conditions and after increasing baseline firing rate (ISI$_B$) by activating endogenous muscarinic receptors with 1 μM muscarine (Lacey et al., 1990) (Fig. 2A-C; compare ISI$_B$ for Con and M). ISI-time plots from a single DA neuron show that activation of muscarinic receptors decreased the response to 100 nS gK$_A$ (Fig. 2B). This trend is evident in the change in ΔISI: muscarinic receptor activation is accompanied by a decrease in the effect of A-type channels (Fig. 2D).

This could reflect a specific effect of muscarinic receptors or be a more general consequence of the change in firing rate. To differentiate between these possibilities, the firing rate was reset toward pre-muscarine levels by injecting negative bias current and then the same dynamic clamp stimulus was applied again (Fig. 2A; compare M to M*). In the continued presence of muscarine, current injection that tended to reverse the muscarinic effect on firing (Fig. 2A-C) also tended to reverse the change in ΔISI (Fig. 2B,D,E). Thus, the change in sensitivity to virtual A-type channels induced by native muscarinic receptors was simply due to the change in pacemaker interval.

This conclusion is further supported by examining the effect of increasing baseline firing rate (ISI↓) with a 10 pA bias current (Fig. 3A). Even though the basis of the decrease in interval was different (i.e., muscarinic receptors were not involved), the
response to virtual A-type channels again was smaller when the pacemaker interval was shortened (Fig. 3A-C). Therefore, the pacemaker interval dependence of the sensitivity to virtual A-type channels is preserved regardless of how firing rate is altered. Furthermore, this relationship, which was first detected by comparing the responses in different DA neurons (Fig. 1), is an intrinsic property of each DA neuron.

The exponential dependence on pacemaker interval applies to voltage-independent cation channels

To test whether the pacemaker control of the effect of A-type voltage-gated K⁺ channels applies to other channels, the response to a non-selective voltage insensitive cation conductance (gCAT) was examined. For this purpose, a 1 nS conductance with a reversal potential (E_{rev}) of 0 mV was added to DA neurons with the dynamic clamp, resulting in increased firing (Fig. 4A,B). Plotting the effect of virtual cation channels on ΔISI of 7 spontaneously firing DA neurons shows that the response retains the exponential dependence on baseline pacemaker interval (Fig. 4C). Therefore, the exponential pacemaker interval relationship applies to channels whether they are voltage sensitive or insensitive, and whether they are excitatory or inhibitory.

DA neuron pacemaker control of responses to virtual excitatory and inhibitory synapses

Because acute responses depend on pacemaker interval (Fig. 1C,D), the effect of episodically active synapses could also be steeply dependent on pacemaker interval. To explore this hypothesis, virtual excitatory synaptic input was applied to DA neurons via the dynamic clamp. Specifically, a template was used in which a 5 nS postsynaptic
nonselective cation conductance \( (g_{SYN_{ex}}, E_{rev} = 0 \text{ mV}) \) was active at a mean frequency of 5 Hz. Furthermore, the template emulated randomly timed synaptic activity to ensure that no phase-cycle bias would be introduced. Turning off the pacemaker by hyperpolarization to -90 mV, which also provides more driving force for the synaptic conductance, showed that this template elicits a 40 s long barrage of small excitatory postsynaptic potentials (Fig. 5A). The dynamic clamp template was then used to excite spontaneously active DA neurons (Fig. 5B, Con). Subsequently, mean baseline firing rate was altered with positive or negative bias current (10 pA), and the identical noisy excitatory barrage was repeated in the same neuron (Fig. 5B, ISI\( \downarrow \) and ISI\( \uparrow \)).

Quantification shows that the response to virtual excitatory synapses increased when ISI\( _B \) was increased and decreased when ISI\( _B \) was lowered (Fig. 5C). Furthermore, analysis of individual responses shows that \( \Delta \text{ISI} \) increased exponentially with ISI\( _B \), the baseline interspike interval (Fig. 5D). The slope in this plot was less than in earlier experiments, but the 95% confidence intervals show that this difference was not statistically significant. Therefore, the efficacy of excitatory ionotopic synapses is subject to exponential pacemaker interval relationship.

The effect of episodic inhibition was then examined. For these experiments, the template of virtual synaptic activity was changed to a hyperpolarizing voltage independent conductance \( (E_{rev} = -65 \text{ mV}) \) so that the episodic inhibitory template slowed spontaneous pacemaker activity (Fig. 6A, Con). As in the previous experiment, the effect of the template was determined after DA neuron firing interval was altered with positive and negative current injection. In 3 neurons in which all 3 conditions were tested (i.e., as in Fig. 6A), increasing ISI\( _B \) amplified the effect of the virtual inhibitory
synapse while decreasing \( \text{ISI}_b \) diminished the perturbation of pacemaking (Fig. 6B). Plotting individual \( \Delta \text{ISI} \) values versus baseline ISI from all experiments shows that the impact of inhibitory virtual synapses was exponentially related to pacemaker interval (Fig. 6C). Hence, the pacemaker control of synaptic efficacy applies to both excitatory and inhibitory virtual synapses.

**Pacemaker control of the efficacy of native metabotropic synapses**

The dynamic clamp can easily emulate simple ionotropic synapses, but applying this approach to complex metabotropic receptor signaling is difficult. Furthermore, the flow of current from the dynamic clamp occurs through the recording pipette, while native transmitter receptors are distributed over the cell body and dendrites. Therefore, to address these limitations, the effect of pacemaker interval on responses to native metabotropic synapses was examined.

For these experiments, the slice was bathed in blockers of ionotropic glutamate and GABA receptors (CNQX, APV and picrotoxin). Then a region of the pars reticulata was subjected to extracellular field stimulation (STIM) at 5 Hz. The timing of this stimulation was controlled by the same randomly distributed template used in dynamic clamp experiments to ensure that phase biases were not introduced. As expected from previous studies, this resulted in slowing of DA neuron pacemaker activity (Fig. 7A, Con). Current clamp recordings revealed that this stimulation evoked an inhibitory response that was altered by the metabotropic GABA\(\beta\) receptor antagonist CGP35348 (CGP) (data not shown) and nearly abolished by a combination of CGP35348 and the metabotropic glutamate receptor (mGluR1) antagonist AIDA (Fig. 7B). Thus, pars
reticulata electrical stimulation activated known native metabotropic inhibitory synapses (Johnson et al., 1992; Fiorillo and Williams, 1998). Because previous experiments predicted that the DA neuron pacemaker interval effect should not depend on the specific mechanisms stimulated, the contributions of other receptors were not studied. Rather, the change in the metabotropic response to pars reticulata stimulation was measured before and after slowing pacemaker rate by injecting -10 pA of bias current. (Fig. 7A, ISI↑).

The ~1.7-fold increase in pacemaker interval caused by current injection increased the synaptic responses ~5.5-fold (Fig. 7A,C). Furthermore, this effect was reversible (Fig. 7C, Con*) showing that the increase in synaptic efficacy is directly dependent on firing rate. Finally, pacemaker interval was reduced by injecting 10 pA of current (Fig. 7A, ISI↓). As expected for the pacemaker interval effect seen in dynamic clamp experiments, the increased firing rate reduced the synaptic response (Fig. 7A,D). Indeed, changing pacemaker intervals ~2.3-fold produced a nearly 9-fold change in the impact of metabotropic inhibitory synaptic stimulation (compare ISI↑ to ISI↓ in Fig. 7D). Thus, DA neuron pacemaker diversity is functionally significant because the response to native metabotropic synapses depends steeply on pacemaker interval.
Discussion

Previous studies have separately examined DA neuron synaptic and pacemaker mechanisms, but the effect of pacemaker rate on DA neuron responses had not been quantified. Therefore, the significance of pacemaker rate diversity and regulation for responses to changes in voltage-gated channels (e.g., Kv4.3 gating and expression regulation by GDNF and D2 autoreceptors, respectively) (Yang et al., 2001; Hahn et al., 2003, 2006) and synaptic conductances were not known. In this study the impact of DA neuron pacemaker rate on responses to dynamic clamp-defined stimuli and metabotropic synapses was determined. First principles suggested that there would be some effect of pacemaker rate, but the finding that known variations in DA neuron pacemaker rate change responses dramatically (>10-fold) regardless of whether they are excitatory, inhibitory, voltage dependent or independent, or sustained or episodic, was not anticipated previously. This exponential effect, which was statistically indistinguishable in all experiments (compare 95% confidence intervals in semilog plots), is expected for homoclinic bifurcation pacemaker models at the transition between repetitive firing and electrical silence, but it is not clear that the relationship applies to small perturbations of ongoing activity that never actually silence the neuron (GB Ermentrout, personal communication). Therefore, both the large magnitude and the mathematical form of the dependence on DA neuron pacemaker interval were not predicted.

Does the DA neuron pacemaker interval relationship, which was discovered in brain slice recordings, apply in the intact brain? Regular pacemaker activity represents only a subset of the activity patterns produced by DA neurons in vivo (Hyland et al.,
However, because the channels that underlie pacemaker activity are present, the more complex and irregular activity seen in vivo is due to interaction of pacemaker mechanisms with ongoing synaptic activity. The data presented in this study suggests that the exponential pacemaker relationship applies to responses to changes in Kv4.3 A-type channel activity, which are induced by GDNF and the antipsychotic drug haloperidol (Yang et al., 2001; Hahn et al., 2003, 2006), metabotropic synapses (e.g., mediated by GABAb and mGluR1 receptors) (Johnson et al., 1992; Fiorillo and Williams, 1998) and ionotropic excitatory and inhibitory synapses that are electrotonically close to the soma (i.e., the site of current injection by the dynamic clamp in this study). On the other hand, the response to distal excitatory synapses that evoke bursts may be more complex. Given that the basis of bursts is not understood, this issue will require more experimentation. However, evoked bursts are not the only relevant change in activity for DA neurons: for example, aversion is linked to inhibition of ongoing activity (Schultz, 2007). Therefore, it is likely that the steep exponential dependence on pacemaker interval applies to many physiological stimuli in vivo.

Because experiments were performed using immature rats, the results presented here do not reveal whether the role of the pacemaker changes during development. It is known that DA neuron mean firing rate increases with age (Tepper et al., 1990), but it is not known whether this reflects a change in background synaptic activity or a remodeling of the pacemaker mechanism itself. A developmental change in background synaptic activity alone would imply that the properties described here apply to the adult. Likewise, a shift in intrinsic pacemaker rate with development would not necessarily alter the exponential dependence on pacemaker interval. With these two
scenarios, pacemaker control of synaptic efficacy could have wide ranging consequences. A high pacemaker rate would serve to make DA neuron activity more autonomous by limiting the impact of many stimuli. Such neurons would produce a tonic unregulated background release of DA. In contrast, a low pacemaker rate would ensure maximal detection of changes in synaptic input resulting in large phasic changes in DA release. Likewise, pacemaker control of synaptic efficacy could also be relevant for antipsychotic drugs that decrease pacemaker interval: the observed ~2 to 3-fold increases in pacemaker interval (Hahn et al., 2003,2006) might have functional benefit because the resultant ~4 to 9-fold increase in synaptic efficacy could compensate for deficits in information processing and attentional functioning associated with schizophrenia (Nuechterlein and Dawson, 1984).

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References


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Legends

**Figure 1.** DA neuron responses to A-type channels exponentially increase with pacemaker interval.  
*A,* Dynamic clamp experiments showing the effect of adding 100 nS A-type potassium conductance (gK_A) for 40 seconds to 3 DA neurons with different intrinsic pacemaker rates. Dashed lines indicate 0 mV.  
*B,* ISI/time plot for the recordings shown in *A.* Note the different y axis scales.  
*C,* Initial ΔISI (ΔISI_i) plotted versus the intrinsic pacemaker interval for 18 individual neurons. The line is a fit to ΔISI= a + exp(k*ISI_B), with a= 0.0081, k = 5.42.  
*D,* Semi-log plot of the data in *C.* Slope of linear regression equaled 1.9, r^2 = 0.78.  
*E,* Steady state ΔISI plotted versus the intrinsic ISI for the same neurons in *C.* The line is a fit to ΔISI= a + exp(k*ISI_B), with a= 0.0024, k = 7.755.  
*F,* Semi-log display of *E.* Slope of linear regression: 1.82, r^2 = 0.66. Dashed lines in *D* and *F* show 95% confidence intervals. Note that log values are base 10.

**Figure 2.** Muscarinic receptors reduce the effect of A-type channels by decreasing pacemaker interval.  
*A,* The effect of 100 nS gK_A (Con) is reduced by 1 μM muscarine bath application (M), but this effect is reversed by resetting ISI_B to the pre-muscarine value with bias current (M*).  
*B,* ISI versus time plots for data in *A.*  
*C,* Mean ISI_B for control (Con), during muscarine application (M) and after adjusting firing rate in muscarine (M*).  
*D,* Quantification of the effects of muscarine and the compensating current injection on ΔISI.  
(E) ΔISI versus ISI_B plot. n = 3 for c-e.
Figure 3. Pacemaker regulation applies to electrically driven changes in the pacemaker rate of individual DA neurons. A, Injection of 50 nS gK_A into a spontaneously pacing DA neuron before (Con) and after increasing its firing rate with +10 pA bias current (ISI ↓). B, ISI plotted versus time for A. Filled diamonds, control; open diamonds, ISI ↓. C, ΔISI versus ISI_B plot for 4 DA neurons.

Figure 4. Pacemaker control of the response to nonselective cation conductance (gCAT). A, Dynamic clamp was used to add 1 nS of gCAT for 60 seconds to a spontaneously firing DA neuron. B, ISI versus time plot for A. C, Relation of log(ΔISI) to ISI_B plotted for 7 cells. Slope: 1.65, r^2 = 0.96. Dashed lines indicate 95% confidence intervals.

Figure 5. Pacemaker control of the response to virtual excitatory synaptic input. A, Dynamic clamp gSYN_ex template (E_rev = 0 mV, g=5 nS, 5 Hz) applied to a DA neuron hyperpolarized to -90 mV. Note that even with the large driving force produced by the holding potential, synaptic responses are small. B, The same gSYN_ex template applied to a spontaneously pacing DA neuron, whose firing rate was altered by bias current: Con, no bias current; ISI ↓, I_{hold}= +10 pA; ISI ↑, I_{hold}= -10 pA. C, ΔISI versus ISI_B plot (n=3). D, The relation of log(ΔISI) and ISI_B is preserved with virtual excitatory synapses: Slope = 1.34, r^2 = 0.80. 95% confidence intervals, which are indicated by dashed lines, indicate that this slope is not significantly different than in Figure 1. Data are from 22 recordings of 10 DA neurons.
**Figure 6.** Pacemaker control of the response to virtual inhibitory synaptic input (gSYN\textsubscript{inh}).  

A, The dynamic clamp template shown in figure 5A was altered by changing \(E_{\text{rev}}\) to -65 mV to generate inhibitory stimulation of spontaneously firing DA neurons. Con, control; ISI\,\downarrow, ISI\subscript{B} is decreased by 10 pA of bias current; ISI\,\uparrow, ISI is increased by -10 pA of bias current.  

B, Quantification of \(\Delta\text{ISI}\) by the inhibitory synaptic template (n=3).  

C, Log(\(\Delta\text{ISI}\)) versus ISI\subscript{B} plot for 21 single experiments performed on 10 spontaneously firing DA neurons treated with gSYN\textsubscript{inh}. Slope: 2.2, \(r^2 = 0.62\). 95% confidence intervals are shown by dashed lines.

**Figure 7.** Pacemaker control of the response to native inhibitory metabotropic synapses.  

A, Stimulation of native inhibitory synapses (STIM) slows DA neuron firing. The same stimulus was applied under control conditions (Con) and after changing firing rate (ISI\,\downarrow or ISI\,\uparrow) with bias current injection. Ionotropic glutamate and GABA receptors were blocked.  

B, IPSP evoked by electrical field stimulation (Control, Con) is reversibly blocked by a combination of GAB\textsubscript{A}b and mGluR1 receptor antagonists (AIDA+CGP). The DA neuron was current clamped to –55 mV.  

C, Increasing ISI\subscript{B} reversibly increased \(\Delta\text{ISI}\) induced by native synapses (n=5). Con\* refers to the resetting of bias current to zero.  

D, The dependence of \(\Delta\text{ISI}\) evoked by metabotropic synapse stimulation on ISI\subscript{B} (n=6).
Figure 1

A

+ gK_A

Neuron 1

Neuron 2

Neuron 3

20 mV

10 s

B

+ gK_A

Neuron 1

ISI = 0.9 s

ΔISI = 1.1 s

Neuron 2

ISI = 0.6 s

ΔISI = 0.3 s

Neuron 3

ISI = 0.4 s

ΔISI = 0.1 s

C

ΔISI_i (s)

ISI_B (s)

D

log ΔISI_i

ISI_B (s)

E

ΔISI (s)

ISI_B (s)

F

log ΔISI

ISI_B (s)
Figure 2

A

+ gK_A

Con

M

M*

20 mV

10 s

B

+ gK_A

ISI (s)

ISI (s)

ISI (s)

20 s

C

ISIB (s)

Con  M  M*

D

ΔISI (s)

Con  M  M*

E

ΔISI (s)

ISIB (s)
Figure 3

A

Con

+ gK_A

ISI_B ↓

20 mV

10 s

B

+ gK_A

ISI (s)

20 s

C

ΔISI (s)

ISI_B ↓

ISI_B (s)
Figure 4

A

B

C

+ gCAT

20 mV

10 s

+ gCAT

20 s

0.0

0.8

1.6

2.4

0.0

0.8

1.6

2.4

-1.0

-0.5

0.0

-1.0

0.0

ISI (s)

log ΔISI

ISI (s)

ISI_B (s)
Figure 5

A

B

+ gSYN

Con

ISI ↓

10 mV

10 s

ISI ↑

C

\[ \Delta \text{ISI} (s) \]

\[ \text{ISI}_B \]

D

\[ \log \Delta \text{ISI} \]

\[ \text{ISI}_B \]
Figure 6

A

+ gSYN\textsubscript{inh}

Con

ISI\textsubscript{B}\downarrow

ISI\textsubscript{B}\uparrow

20 mV

10 s

B

\[ \Delta \text{ISI} (s) \]

C

\[ \log \Delta \text{ISI} \]

\[ \text{ISI}_B (s) \]
Figure 7

Panel A:
- **Con**: Baseline activity.
- **$\text{ISI}_B \uparrow$**: Increase in $\text{ISI}_B$.
- **$\text{ISI}_B \downarrow$**: Decrease in $\text{ISI}_B$.
- STIM: Stimulation period.

Panel B:
- **Con**: Control condition.
- **AIDA+ CGP**: Application of AIDA+ CGP.
- **wash**: Washout period.

Panel C:
- $\Delta\text{ISI}$ bar graph showing changes in $\text{ISI}$ under different conditions.

Panel D:
- $\Delta\text{ISI}$ against $\text{ISI}_B$ graph illustrating the relationship between $\text{ISI}_B$ and $\Delta\text{ISI}$.

Key: STIM = Stimulation; $\text{ISI}_B$ = Intracellular spike interval.