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ORIGINAL ARTICLE

Two Types of Burst Firing in Gonadotrophin-Releasing Hormone Neurones

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Gonadotrophin-releasing hormone (GnRH) neurones fire spontaneous bursts of action potentials, although little is understood about the underlying mechanisms. In the present study, we report evidence for two types of bursting/oscillation driven by different mechanisms. Properties of these different types are clarified using mathematical modelling and a recently developed active-phase/silent-phase correlation technique. The first type of GnRH neurone (1–2%) exhibits slow (~0.05 Hz) spontaneous oscillations in membrane potential. Action potential bursts are often observed during oscillation depolarisation, although some oscillations were entirely sub-threshold. Oscillations persist after blockade of fast sodium channels with tetrodotoxin (TTX) and blocking receptors for ionotropic fast synaptic transmission, indicating that they are intrinsically generated. In the second type of GnRH neurone, bursts were irregular and TTX caused a stable membrane potential. The two types of bursting cells exhibited distinct active-phase/silent-phase correlation patterns, which is suggestive of distinct mechanisms underlying the rhythms. Further studies of type 1 oscillating cells revealed that the oscillation period was not affected by current or voltage steps, although amplitude was sometimes damped. Oestradiol, an important feedback regulator of GnRH neuronal activity, acutely and markedly altered oscillations, specifically depolarising the oscillation nadir and initiating or increasing firing. Blocking calcium-activated potassium channels, which are rapidly reduced by oestradiol, had a similar effect on oscillations. Kisspeptin, a potent activator of GnRH neurones, translated the oscillation to more depolarised potentials, without altering period or amplitude. These data show that there are at least two distinct types of GnRH neurone bursting patterns with different underlying mechanisms.

Key words: burst, oscillation, hypothalamus, neuroendocrine, parabolic.

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Gonadotrophin-releasing hormone (GnRH) neurones form the final common pathway by which the central nervous system regulates fertility. GnRH is released in an episodic manner in males and during most of the female reproductive cycle (1–5). Immortalised GnRH neurones exhibit episodic release, suggesting this patterning may be intrinsic to GnRH neuronal networks (6–8). More recent studies of identified GnRH neurones in brain slices and primary cultures demonstrated episodic activity in both action potential firing and intracellular calcium levels (9–12). These biophysical observations revealed that episodic activity is observed not only at intervals consistent with that of hormone release *in vivo* (which occurs at intervals from once every several minutes to once every several hours depending on reproductive state) (13,14), but also at much higher frequencies. The highest frequency biophysical activity observed in GnRH neurones thus far is the clustering of action potential firing into bursts. Burst firing is important because it has been shown to increase the efficacy of neuropeptide release and neurotransmission in other systems (15,16). The interval between bursts of firing in GnRH neurones varies within and among cells; peaks in the mean firing rate of individual GnRH neurones occur at intervals that are similar to what would be expected for GnRH release and are associated with bursts being closer together (10,17), suggesting an association between changes in burst interval and hormone secretion.

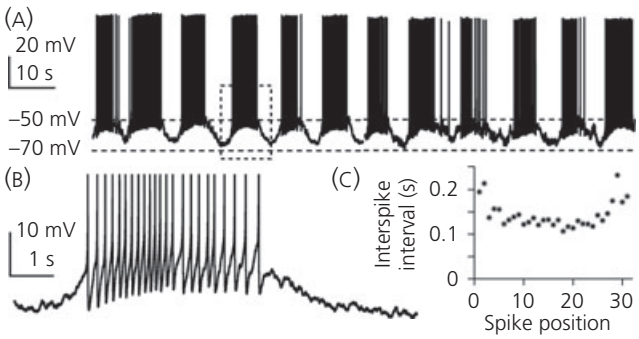

Fig. 1. Parabolic burst firing in a gonadotrophin-releasing hormone (GnRH) neurone. (A) Example of regular parabolic bursting in a GnRH neurone recorded in current-clamp mode. (B) Expansion of area in box. (C) Plot of interspike interval versus spike position.

Burst firing in neurones and excitable endocrine cells can be driven by a variety of intrinsic factors, as well as extrinsic or network factors (18). In the present study, we report evidence for two distinct types of bursting in GnRH neurones. One type is irregular in duration and periodicity. The other type is much more regular and exhibits a parabolic pattern within the interspike interval (Fig. 1). This latter type of bursting, called 'parabolic bursting', has been observed in other neurones, most notably the R15 neurone of the mollusk *Aplysia*, where it was first described and remains a classic example of parabolic bursting (19,20). A characteristic feature of this type of bursting is the existence of two or more slow processes, which combine to generate an underlying periodic slow rhythm. This rhythm can persist even in the absence of action potentials (21) and, even if action potentials are present, the parabolic interspike interval pattern can be difficult to discern (22). Using a well-known mathematical model of the R15 bursting neurone developed by Plant (23), we illustrate that this type of bursting oscillation can be identified by the correlation pattern between active (up state) and silent (down state) phases, even if impulses are inhibited by blocking sodium channels. We show that parabolic oscillations are intrinsically generated and rapidly altered by oestradiol, a critical physiological feedback mediator (24), and by kisspeptin, a strongly activating neuromodulator of GnRH neurones (25,26).

Materials and methods

Animals

Adult (2–4 months old) transgenic mice in which green fluorescent protein (GFP) is genetically targeted to GnRH neurones were used (27). Mice were housed under a 14 : 10 h light/dark cycle (lights off 16.30 h EST) and fed Harlan 2916 chow (Indianapolis, IN, USA) and water *ad lib.* All procedures were approved by the University of Virginia Animal Care and Use Committee or the University of Michigan University Committee on the Use and Care of Animals.

Because only a small subpopulation of GnRH neurones exhibit parabolic oscillations, studies on this type of activity were conducted whenever encountered in animals that had been prepared for other experiments. A total of 26 oscillating cells were recorded over approximately 5 years of data collection. During that time, approximately 1500 cells were recorded in the whole-cell configuration; the percentage of oscillating GnRH neurones is thus approximately 2%. The majority of animals were females ovariectomised (OVX) 5–9 days previously (n = 20 animals). OVX females treated with physiological levels of oestradiol (n = 1), intact dioestrous female (n = 1) and castrate males (n = 1) were also studied. One (n = 20 mice) or two (n = 3 mice) cells were studied per animal. The low number of cells in all but OVX mice precludes statistical comparison among models; the oscillation period was very similar and data have been combined. All irregularly bursting cells were recorded in slices prepared from OVX mice.

Slice preparation

Reagents were purchased from Sigma Chemical Company (St Louis. MO, USA) unless noted otherwise. Sagittal brain slices were prepared as reported previously (28) with slight modifications (29). Briefly, mice were decapitated and the brain was rapidly removed and placed in ice-cold high-sucrose saline solution containing (in mM): 250 sucrose, 3.5 KCl, 26 $NaHCO_3$, 10 glucose, 1.25 NaH_2PO_4, 1.2 $MgSO_4$ and 3.8 $MgCl_2$. Sagittal (300 μm) slices were cut with a Vibratome 3000 (Ted Pella, Inc., Redding, CA, USA). Slices were incubated in a 1 : 1 mixture of sucrose saline and artificial-cerebrospinal fluid (aCSF) containing (in mM): 125 NaCl, 3.5 KCl, 26 $NaHCO_3$, 1.25 NaH_2PO_4, 2.5 $CaCl_2$, 1.2 $MgSO_4$ and 10 D-glucose (pH 7.4) for 30 min at 31 °C and then transferred to 100% aCSF and incubated for at least an additional 60 min at room temperature (22–24 °C); all slices were used within 6 h of preparation.

Recordings

For recording, slices were placed in a chamber on the stage of an Olympus BX51WI upright fluorescent microscope (Olympus, Tokyo, Japan) and continuously superfused at 5–6 ml/min with oxygenated aCSF at 30–32 °C. Slices were stabilised in the chamber for ≥ 5 min before recording. GFP-GnRH neurones were identified by brief illumination at 470 nm. Recordings were performed using an EPC 10 double amplifier running PATCHMASTER software (HEKA Electronics, Lambrecht, Germany). The location of each GnRH neurone studied was mapped onto figures obtained from a mouse brain atlas (30); however, no consistent location or gross anatomical feature was noted as an identifying characteristic for oscillating cells. Basic electrophysiological characteristics of both oscillating and irregular bursting GnRH neurones were monitored and compared; no differences were noticed in any parameter monitored between these two types of cells (n = 9–14 cells of the 26 total oscillating cells per parameter; Table 1).

For whole-cell recordings, recording pipettes (2–3 MΩ) were filled with a solution containing (in mM): 125 K gluconate, 20 KCl, 10 HEPES, 5 ethylene glycol tetraacetic acid (EGTA), 4.0 MgATP, 0.4 NaGTP, 1.0 $CaCl_2$ (pH 7.3), 300 mOsm (n = 22 oscillating cells and all irregular bursting cells) or 140 KCl, 10 HEPES, 5 EGTA, 4.0 MgATP, 0.4 NaGTP and 1.0 $CaCl_2$, pH 7.3, 290 mOsm (n = 4 cells). Calculated (31) liquid junction potential of 13 mV for the K gluconate-based solution and 3 mV for the KCl-based solution were not corrected.

Drug treatments

Treatments were bath applied and varied among cells; details are in the results. 20 μM bicuculline methiodide, 20 μM D(–)2-amino-5-phosphonovaleric acid (APV) and 10 μM 6-cyano-7-nitroquinoxaline were used to block $GABA_A$, NMDA and AMPA/KA receptors, respectively. Tetrodotoxin (TTX; 0.5 μM; Calbiochem, San Diego, CA, USA) was used to block fast sodium channels. Small conductance (SK) calcium-activated potassium current (I_{KCa}) was blocked with 200 nM apamin; large conductance (BK) I_{KCa} was blocked with 100 nM iberiotoxin. The G-protein coupled oestradiol receptor GPR30 (32) was activated with the G1 agonist (100–200 μM). The effects of oestradiol (10–100 nM) and kisspeptin (10 nM; Phoenix Pharmaceuticals, Inc., Belmont, CA, USA) were also studied.

Table 1. Electrophysiological Characteristics of Oscillating Versus Irregular Bursting Gonadotrophin-Releasing Hormone Neurones.

Parameter	Oscillating (n = 9–14)	Irregular (n = 9–13)
Input resistance (MΩ)	905 ± 54	876 ± 22
Action potential amplitude (mV)	80.2 ± 2.5	78.9 ± 1.3
Afterhyperpolarising potential amplitude (mV)	32.2 ± 0.7	31.0 ± 0.8
Action potential width at half maximal amplitude (ms)	1.1 ± 0.03	1.1 ± 0.04
Action potential threshold (mV)	−32.2 ± 1.3	−31.9 ± 0.6
Resting membrane potential (mV)	−61.9 ± 1.0	−60.7 ± 1.2

Values are the mean ± SEM. P > 0.3 for all comparisons via an unpaired t-test. Action potential amplitude is measured from prespike potential; afterhyperpolarisation amplitude is measured from the threshold. Note that not all properties could be monitored in all 26 oscillating cells because some were quiescent or had action potentials pharmacologically blocked; initial resting membrane potential (I = 0) could not be determined in cells with high-amplitude oscillations; and some cells were observed without imposing voltage steps that are needed to monitor input resistance.

Correlation analysis

To analyse the relationship between burst active and silent phases for experimental and simulation data, we first had to define these phases (Fig. 2A,D). After visual inspection of a trace, we define two parameters, V_t (mV) and δ_t (ms) (in most cases $V_t = -30$ mV and $\delta_t = (200,1000)$ ms). When the voltage, V, is greater than V_t, a spike is recorded. Once all the spikes in a trace are recorded, if two adjacent spikes are more that δ_t apart, they are not considered part of the same burst. A solitary spike is one that is at least δ_t apart from the preceding and following spike. It follows that the shortest active phase duration is that of spike, whereas the shortest silent phase duration is that between a burst and an adjacent solitary spike. Because subthreshold oscillations and those in the presence of tetrodotoxin do not have action potentials, a different approach was used. We first normalised each voltage trace to lie between 0 (minimum) and 1 (maximum). We defined the 'active phase' as the time during which the normalised voltage is > 0.5, and the 'silent phase' as the time during which the normalised voltage is below 0.5. Once active and silent phases were assigned (for bursting or subthreshold oscillations), we then constructed a pair of scatter plots. In one scatter plot, the duration of a burst active phase is plotted against the duration of the immediately preceding silent phase (Fig. 2). This is varried out for each burst in the voltage trace, producing a scatter plot of points. In the other scatter plot, the burst active phase duration is plotted against the immediately following silent phase duration. Thus each burst is represented by a point in each of the two scatter plots. We then compute the Pearson correlation coefficient of the scatter plots. We have shown previously that the correlations between active and silent phases are indicators of the dynamical mechanism underlying relaxation oscillations and bursting (33). That is, different types of bursting patterns exhibit different correlation patterns. We use this technique in the present study to discriminate between the parabolic bursting observed in some GnRH neurones and the irregular (nonparabolic) bursting observed in others.

Mathematical modelling

We used a well-known model for parabolic bursting that was developed for the R15 neurone of the mollusk *Aplysia* (23) to illustrate properties that are characteristic of parabolic bursting. In particular, we use the model to demonstrate that parabolic bursting oscillations have a negative correlation between the active and the next silent phase duration, even if spikes are inhibited by blocking sodium channels (in which case 'active phase' means the up state of the small voltage oscillation that persists when spikes are blocked).

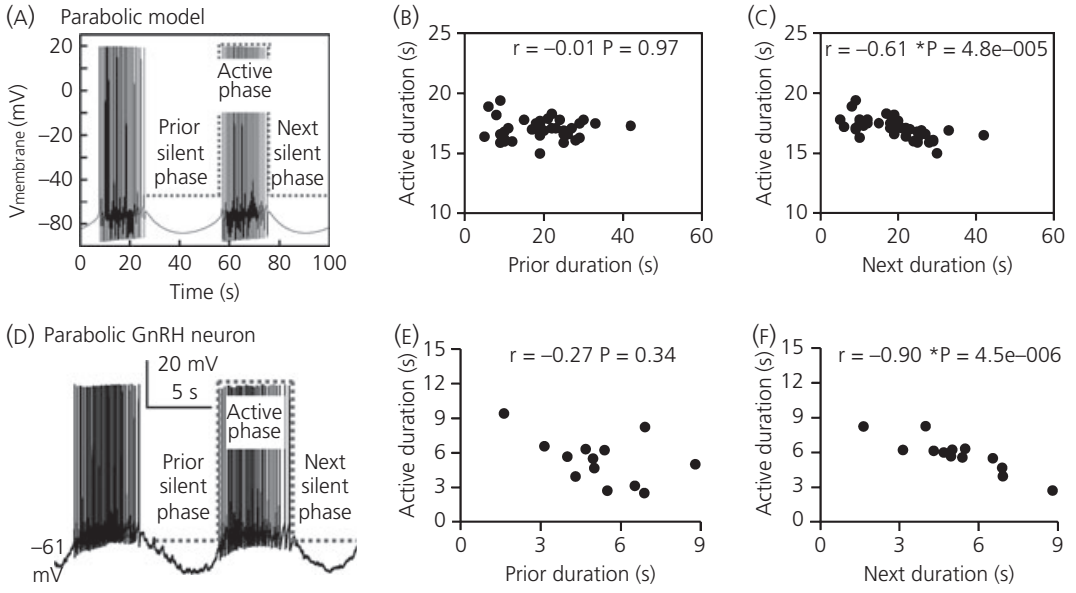

Fig. 2. Active and silent period correlations in a model parabolic neurone and a gonadotrophin-releasing hormone (GnRH) neurone. (A) Plant model for parabolic bursting defining prior silent phase, active phase and next silent phase. (B, C) Scatter plots for prior silent duration versus active duration (B) and next silent duration versus active duration (C) showing significant correlation between next silent duration and active duration. (D) Parabolic bursts in a GnRH neurone illustrating phases. (E, F) Scatter plots for prior silent duration versus active duration (E) and next silent duration versus active duration (F) showing significant correlation between next silent duration and active duration.

In addition to the original publication, the Plant model is described on the *Scholarpedia* web site (http://www.scholarpedia.org/Plant_model). It is a Hodgkin–Huxley-type model with a leak current, spike-producing ionic currents and two slowly-activating ionic currents that package the spikes into bursts, $C_m \frac{dV}{dt} = -\left(I_{in,fast} + I_{out,fast} + I_{in,slow} + I_{out,slow} + I_L\right)$ where $I_{in,fast}$ and $I_{in,slow}$ are fast- and slowly-activating inward currents, respectively (with Nernst potential of 30 mV), and $I_{out,fast}$ and $I_{out,slow}$ are outward K^+ currents (with Nernst potential of −75 mV). The leakage current, I_L, has a constant conductance and a reversal potential of −40 mV. In addition to the voltage differential equation, there is a differential equation for inactivation of $I_{in,fast}$, one for activation of $I_{in,slow}$, and one for activation of $I_{out,fast}$. Finally, there is a differential equation for the free cytosolic Ca^{2+} concentration, which activates $I_{out,slow}$. Parameter values used are the same as those used for Fig. 1 (http://www.scholarpedia.org/Plant_model). Computer simulations were performed with the XPPAUT software package (34) using the Forward Euler method. The code is available as freeware from http://www.math.f-su.edu/~bertram/software/neurone.

Analysis

Stored data traces were analysed offline using IGOR PRO (WaveMetrics, Portland, OR, USA). Data were transferred to EXCEL (Microsoft Corp., Redmond, CA, USA), or INSTAT (Graph Pad Software, San Diego, CA, USA) for statistical analysis. Summary data are shown as the mean ± SEM.

Results

Burst firing and membrane oscillations in GnRH neurones

GnRH neurones in slices, after isolation and in primary and immortalised cultures, exhibit burst firing over a range of periods (9,17,28,35–38). The interval between bursts varies widely in long-term targeted extracellular recordings (28,39–41). Most GnRH neurones exhibit burst firing without periodic plateaus in the membrane potential. However, some cells (approximately 2%) produce profound periodic plateaus in the membrane potential (e.g. AMPA, NMDA and $GABA_A$ receptors are blocked; Fig. 1A). Action potential firing can be observed on the peaks of some of these oscillations, whereas others are completely subthreshold (e.g. pre-treatment; Fig. 7A). Figure 1(B) shows an expansion of the oscillation peak, or upstate, indicated by the dashed box in Fig. 1(A). This cell fires spikes for almost 4 s, with the interspike interval first declining and then increasing (Fig. 1C). This parabolic trend in the interspike interval is the defining characteristic of a parabolic bursting pattern that has been observed in other neurones (42,43).

Although parabolic bursting is often characterised by a clear parabolic distribution of interspike intervals as in Fig. 1(C), this feature may be subtle and not evident (22). Also, the determination of a parabolic distribution of interspike interval is of no use in cases when spiking does not occur (e.g. when sodium channels are blocked). A better way to characterise this form of bursting is in terms of the underlying dynamics (22,42). Parabolic bursting oscillations are a result of the interaction of two slow processes (such as channel activation/inactivation) that is capable of producing a rhythm even in the absence of electrical impulses (23). This interaction of slow processes also produces a characteristic pattern between active (or up state) and silent (or down state) phases of the bursting (or subthreshold) oscillation, as we demonstrate in Fig. 2 using a classic mathematical model for parabolic bursting (23). Figure 2(A) shows two bursts produced by the Plant model, with a superimposed envelope defining the active or spiking phase of the burst and the prior and next silent phases. This envelope extends throughout the voltage trace (n = 36 bursts) and serves to identify silent versus active phases, allowing computation of their respective durations. Figure 2(B) shows, for each burst, the active phase duration plotted against the immediately prior silent phase duration. Each point in the scatter plot corresponds to a burst and its prior silent phase (i.e. 36 points corresponding to 36 bursts). There is no significant correlation between active and prior silent phases ($P = 0.97$, $n = 36$). In the scatter plot of Fig. 2(C), each burst active phase is plotted against the immediately following silent phase. In this case, there is a significant negative correlation between the two phases ($r = -0.61$, $P < 0.01$, $n = 36$). Thus, the duration of the prior silent phase of the burst does not predict the length of the burst active phase, although the duration of the active phase does predict the next silent phase. For example, a long active phase predicts the silent phase that follows will be short. This pattern of correlations contrasts with the pattern we have observed for other types of bursting oscillations (33), such as the 'square-wave bursting' that is exhibited by pancreatic islets and pre-Bötzinger neurones.

We next perform the same correlation analysis on a voltage trace from a GnRH neurone exhibiting parabolic bursting (Fig. 2D, only two bursts are shown). As with the model neurone, there is no significant correlation between active and prior silent phase durations ($P = 0.34$, $n = 13$; Fig. 2E). However, there is a significant negative correlation between active and next silent phase durations ($r = -0.90$, $P < 0.01$, $n = 13$; Fig. 2F). This correlation pattern is a characteristic of parabolic bursting, and serves as a fingerprint for the slow dynamics underlying this type of spiking pattern.

A second pattern of burst firing is observed in a majority of GnRH neurones. This consists of irregular bursts of action potentials with no obvious parabolic pattern in the interspike intervals (Fig. 3A). These irregular bursts are of varying duration (Fig. 3B) and there is no parabolic relationship between spike position and interspike interval, and only a very mild spike frequency accommodation is observed (Fig. 3C). When the same correlation analysis is performed on irregular bursting GnRH neurones, no significant correlation is observed between active phase duration and either prior or next silent phase duration (Fig. 3D), further illustrating that the underlying dynamics are different from those of parabolic bursting GnRH neurones.

GnRH neurone membrane potential oscillations are intrinsically generated

We next examined the effects of blocking fast sodium channels required for action potential spikes with TTX on each type of bursting activity. GnRH neurones exhibiting parabolic bursting (n = 3) were recorded before and after addition of TTX; blockers for AMPA, NMDA and $GABA_A$ receptors were present throughout the recording to further isolate the cell from fast synaptic

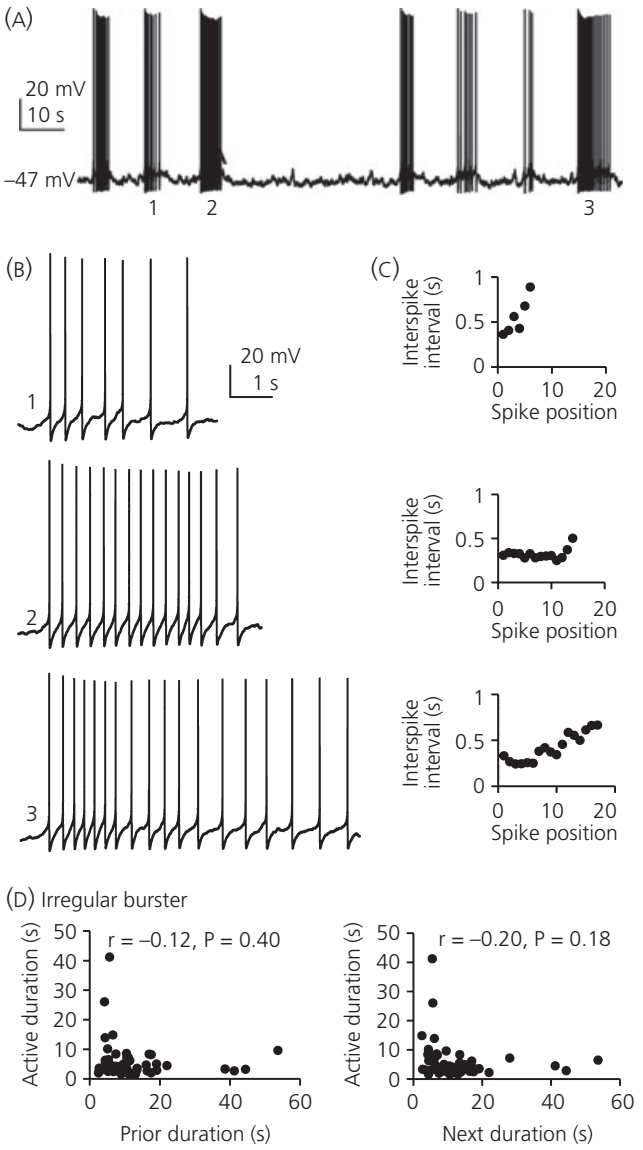

Fig. 3. Irregular burst firing in a gonadotrophin-releasing hormone (GnRH) neurone. (A) Example of irregular bursting in a GnRH neurone recorded in current-clamp mode. (B) Expansion of three bursts indicated in (A). (C) Plot of interspike interval versus spike position for these three bursts. (D) Scatter plots for prior silent duration versus active duration (left) and next silent duration versus active duration (right) showing no significant correlation.

transmission. Despite blocking spikes, membrane oscillations persisted (Fig. 4A). Correlation analysis revealed that the significant negative correlation between active phase duration and next silent phase duration was maintained in the absence of spikes (n = 35; Fig. 4B), suggesting that the mechanism for the membrane oscillations in the presence of TTX is the same as that for the periodic oscillations underlying parabolic bursting. Furthermore, TTX did not alter the oscillation period (control 17.8 ± 0.3 s, TTX 17.9 ± 0.8 s, n = 2) or amplitude (control 44.4 ± 6.2 mV, TTX 39.5 ± 8.2 mV). The persistence of these oscillatory plateau potentials in the presence of TTX suggests that they are intrinsically generated and also that the mechanism does not involve a TTX-sensitive sodium conductance. By marked contrast, TTX treatment of irregularly bursting neurones resulted in a stable membrane potential (Fig. 4C), despite a similar period of burst firing prior to treatment. As in Fig. 3, no significant correlations were observed between active phase duration and either prior or next silent phase duration (analysis performed only during the control period when the phases could be defined, n = 81; Fig. 4D).

Neither electrical, nor chemical synaptic transmission detected between GnRH neurones

The data above suggest oscillations are intrinsic to GnRH neurones; however, recent work in another neuroendocrine system (44) indicated that gap junctional communication was important in the oscillations of those cells. We examined pairs of irregularly bursting GnRH neurones that appeared to contact one another based on the GFP fluorescent signal in the slice for evidence of either electrical coupling (n = 26 pairs; Fig. 5A) or chemical coupling (n = 19 pairs; Fig. 5B,C). There was no evidence of either type of coupling in the pairs examined, similar to a recent report in which no electrical coupling was observed between pairs of GnRH neurones (45). In two additional cases of recorded pairs of GnRH neurones, one cell of the pair exhibited membrane oscillations, whereas the other did not. Furthermore, there was no marked difference in the frequency of GABAergic postsynaptic currents based on oscillation phase (up state 1.2, 0.4, 0.2 Hz postsynaptic currents, down state 1.1, 0.4, 0.2 Hz postsynaptic currents, respectively, for n = 3 cells; note that these three cells were recorded with 140 mM KCl pipette solution).

Voltage dependence of parabolic oscillations

Persistent oscillations were recorded in GnRH neurones in both voltage- and current-clamp modes. These recording modes have different conventions; a depolarising response recorded in current-clamp is observed by a change towards less negative membrane potentials, whereas a change in membrane current that would have a hyperpolarising effect on membrane potential is shown as increasingly negative current. To be consistent in examining data from these two modes, we have adopted the term upstate to refer to what would be an oscillation peak in current-clamp (a nadir in voltage-clamp) and downstate to refer to the nadir of a current-clamp recording (peak in voltage-clamp). This is illustrated in a representative current-clamp (Fig. 6A) and representative voltage-clamp recording (Fig. 6B) with the up and down states labelled.

Because the oscillations underlying parabolic bursting appear to be intrinsically generated, we examined the effect of manipulating membrane potential in two ways. First (n = 3 cells), continuous direct current was injected to either hyperpolarise or depolarise the membrane for 3-min periods (Fig. 6C). These manipulations had no effect on oscillation period (Fig. 6D) but some damping of amplitude was observed with both positive and negative current injection (Fig. 6E), possibly attributable to reduced driving force. Second (n = 2 cells), 10-s duration voltage steps were made in voltage clamp (from −60 mV to either −20 mV or −100 mV; Fig. 6F,G).

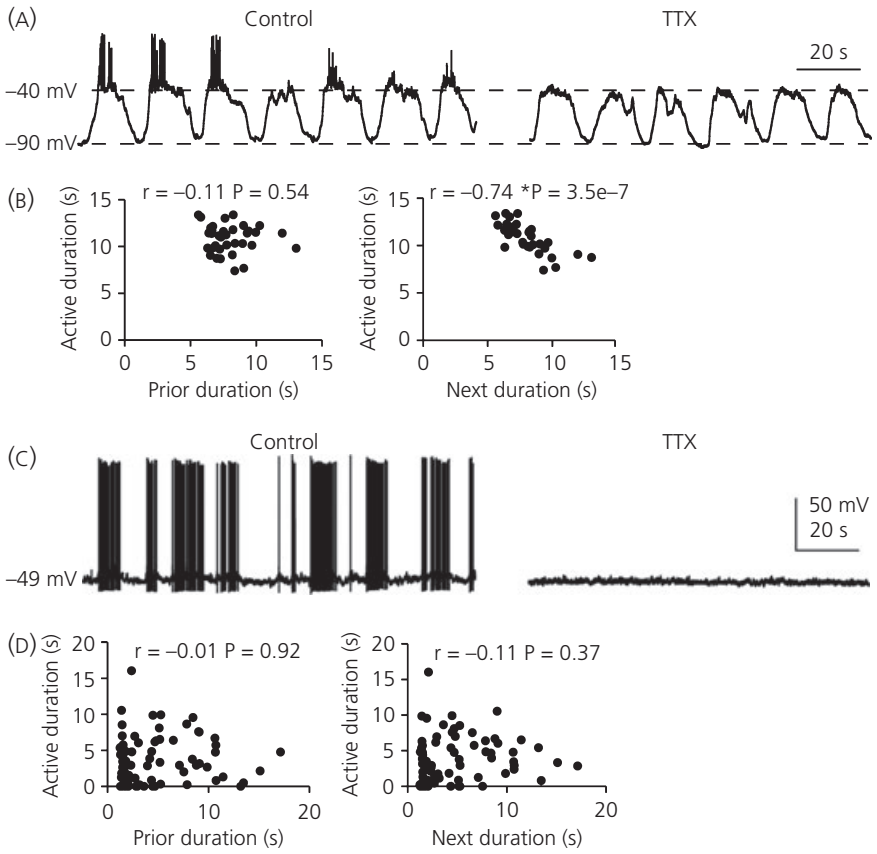

Fig. 4. A slow rhythm underlies bursting in one type of bursting neurone, but not the other. (A) Oscillating gonadotrophin-releasing hormone (GnRH) neurone recorded during application of tetrodotoxin (TTX) before full effect (left) and after full effect of the drug (right). Spikes are blunted in amplitude in left panel attributable to initial phases of drug action. Oscillations persist after spikes are blocked. (B) Scatter plots for prior silent duration versus active duration (left) and next silent duration versus active duration (right) showing significant correlation between next silent duration and active duration. (C) Irregular bursting GnRH neurone under control conditions (left) and after TTX (right). No oscillations in membrane potential become evident after spikes are blocked. (D) Scatter plots for prior silent duration versus active duration (left) and next silent duration versus active duration (right) showing no significant correlation.

Comparisons were made by averaging amplitude and period for the three oscillations just before and just after the step. Neither hyperpolarising, nor depolarising voltage steps had any effect on the oscillation period or amplitude regardless of phase of the oscillation when the step was applied (Fig. 6H,I). The phase shift of the next oscillation peak following the perturbation relative to the expected time (based on the mean period) is shown in Fig. 6(J). In all cases, oscillation peaks occurred within 0.5 s of the expected time (< 1 SD), indicating that the voltage step had little impact on the intrinsic pacemaker oscillation.

Oestradiol rapidly alters membrane oscillations and firing pattern of GnRH neurones

To begin to understand how these oscillations might be physiologically regulated, we examined the effect of a rapid application of oestradiol (10 nM), an important regulator of GnRH neurone function. Oestradiol had marked effects on several aspects of this oscillation (Fig. 7). The period of the rhythm was not altered ($P > 0.2$; Fig. 7B), whereas the amplitude was reduced ($P < 0.05$; Fig. 7C). Oestradiol appeared to particularly block mechanisms involved in the hyperpolarising phase of the oscillation; the peak was unchanged ($P > 0.2$), whereas the nadir of the oscillation was depolarised ($P < 0.05$; Fig. 7C). When oestradiol was present, many of the oscillations evoked action potentials. Unlike oestradiol, the putative membrane oestradiol receptor agonist G1 had no effect (n = 2, data not shown). Interestingly, re-examination of previously published data (46) revealed that even in irregularly bursting cells, acute oestradiol treatment often produced a burst firing pattern that, although not parabolic, had a similar period to oscillations (burst onset to burst onset 15.9 ± 0.5 s, spikes/burst 9.4 ± 1.2, n = 26 cells). This indicates the phenomenon of rhythmic bursting activity in GnRH neurones with a period between 10 and 20 s occurs even in irregularly bursting cells.

Previous work from our laboratory had established calcium activated potassium channels as one possible target for rapid oestradiol action (46). We thus examined the effects of the BK blocker iberiotoxin and the SK blocker apamin. Figure 7(D) shows an example current-clamp recording in which these were applied separately, indicating the relative lack of effect of iberiotoxin on the oscillation. Although apamin blunted the amplitude (Fig. 7E), it had no effect on frequency (Fig. 7F). Addition of apamin with

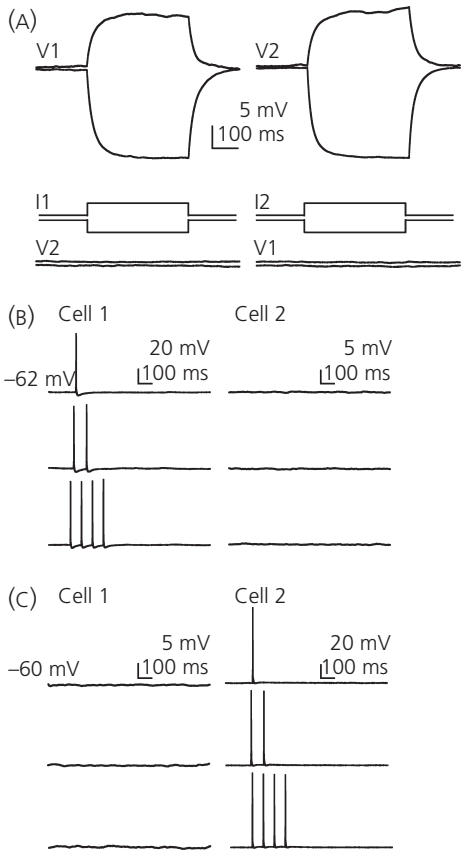

Fig. 5. No electrical or fast chemical coupling detected between gonadotrophin-releasing hormone neurones. (A) Membrane response (top) of cells 1 (V1) and 2 (V2) to +15 to −40 pA current injection (I1, I2, respectively). Bottom, membrane potential of non-injected cell, 100 traces averaged. (B, C) Elicitation of 1, 2 or 4 spikes by brief current injections (300 pA, 3 ms) into cell 1 fails to elicit postsynaptic potentials in cell 2; identical injections into cell 2 fail to elicit postsynaptic potentials in cell 1 (mean of 50 traces).

iberiotoxin depolarises the downstate of the oscillation at the same time as having minimal effect on the upstate, similar to oestradiol (Fig. 7G). Taken together, these observations suggest that one possible mechanism for the rapid action of oestradiol on the oscillation is reduced conductance via small conductance potassium channels.

Kisspeptin translates the entire oscillation towards the upstate

Kisspeptin is a strong activator of GnRH neurones (25). We thus examined its effects on the oscillation. In both current- and voltage-clamp (n = 2 each, cell VC2 recorded with 140 mM KCl pipette solution), kisspeptin translated the oscillation towards the upstate at the same time as having no effect on amplitude or period (Fig. 8A–C). These data suggest another possible level of oscillation in GnRH neurones in which neuromodulators initiate mechanisms that move upstates in the intrinsic oscillation nearer and further from the action potential threshold, and thus generate longer term patterns in the firing rate.

Discussion

In the present study, we provide evidence for two distinct types of bursting in GnRH neurones with distinct underlying mechanisms. One type is irregular in duration and periodicity. The other is very regular, but rare, and exhibits a parabolic interspike interval trajectory.

Parabolic bursting has been observed in other neurones, most notably the R15 neurone of the mollusk *Aplysia*, where it was first described (19,20). One feature of parabolic bursting in R15 is that blocking action potential firing does not necessarily eliminate the plateau phases of the membrane potential oscillation (21), a feature that was explained using a mathematical model (47). In GnRH neurones exhibiting parabolic bursting, we find that similar oscillation plateaus persist when tetrodotoxin is present, in contrast to irregular bursters in which membrane potential is very stable after blockade of action potential firing. Intriguingly, we find that both the period of oscillations and the up-phase/down-phase correlation patterns in cells not exhibiting action potentials are similar to that seen in the GnRH neurones exhibiting parabolic bursting. This suggests that currents generating the slow rhythm are responsible for the parabolic bursting pattern and that this oscillation may have a pacemaker function.

We introduced a new technique to identify parabolic bursting and distinguish it from the irregular bursting seen in many other GnRH neurones. The well-known Plant model was used to demonstrate that parabolic bursting gives rise to no correlation between the active phase and the prior silent phase, although a negative correlation between the active phase and the following silent phase. This is true (not shown) even for the simpler model of parabolic bursting developed by Baer *et al.* (48), and is a property of parabolic bursting and not specific to the model itself. This pattern is unlike that of the irregular bursting neurones and unlike that of other forms of bursting such as square-wave or elliptic bursting (33). The correlation pattern thus serves as a 'fingerprint' for parabolic bursting, and persists even when impulses are blocked.

In oscillating GnRH neurones, the period of the oscillation was remarkably robust, consistent with pacemaking. Period was not altered by continuous direct current injection, nor by shorter duration voltage-steps or current injections, nor by the drug treatments that were tested. The period of GnRH neurone oscillations is quite long compared to other central neuroneal oscillations; for example, theta-frequency oscillations (3–8 Hz) in rodent hippocampus and entorhinal cortex (49,50) or oscillations associated with burst firing in neocortical neurones (around 0.5–1 Hz) (51–54). Interestingly, the period in GnRH neurones is very similar to that of dopaminergic (TIDA) neurones of the arcuate nucleus, which are largely responsible for the control of prolactin release (44). Despite this similarity in period, these two neuroendocrine cell types appear to use different mechanisms as the TIDA neurone rhythm is abolished by tetrodotoxin, whereas that in GnRH neurones is not. In TIDA neurones, network properties and interconnections, including changes in GABAergic transmission from up to down state and gap junctional communication, were critical in sustaining the oscillation, whereas, in GnRH neurones, the oscillation appears to be intrinsically generated because it occurs in the presence of tetrodotoxin

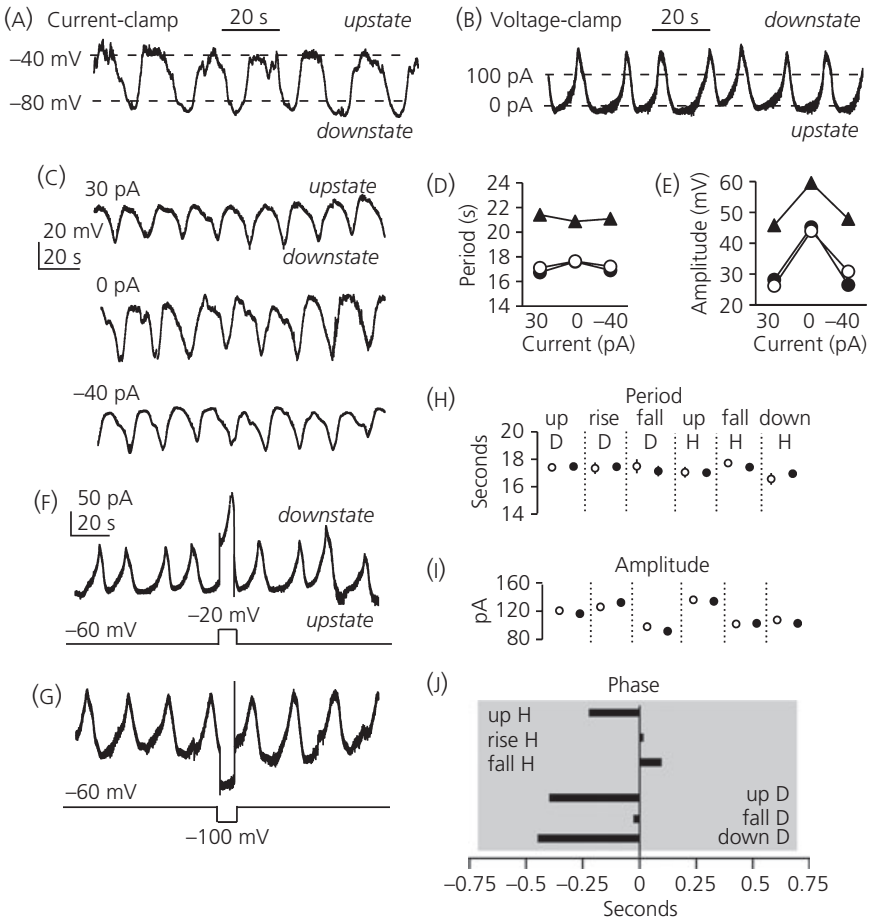

Fig. 6. Effect of membrane potential and membrane current changes on pacemaker oscillations. (A, B) Current-clamp (A) and voltage-clamp (B) recordings of oscillating gonadotrophin-releasing hormone neurones defining up and down state (in italics on right of each panel). (C) Current-clamp recording showing that injecting steady-state DC current to alter membrane potential does not affect oscillation period (D), but does dampen amplitude (E). Different symbols in (D) and (E) are individual cells. (F, G) Voltage-clamp recording showing that 10 s depolarising (F) or hyperpolarising (G) steps do not affect oscillation period or amplitude. (H–J) Analysis of the cell in (G) showing that application of the 10-s voltage step in either depolarising (D) or hyperpolarising (H) directions at different phases (up, fall, down, rise) of the oscillation does not alter period (H), amplitude (I) or phase of the next peak after the step (J). In (H) and (I), the white symbol is the mean of three oscillations before the step and the black symbol is the mean of three oscillations after the step. Different step trials are separated by vertical dashed lines. In (J), the predicted time of the next oscillation peak is show at 0 s on the x-axis, and the actual time is shown by the black bars. The grey box shows 1 SD from the mean period. Upstate and downstate for the different recording modes are indicated on right side of each panel in italics. All recordings were made in the presence of tetrodotoxin and blockers of fast synaptic transmission.

and blockers of fast synaptic transmission. Furthermore, no evidence of chemical or electrical synapses were detected between GnRH neurone pairs.

The mechanisms underlying parabolic bursting in GnRH neurones are unresolved; however, there are some mechanisms that can be ruled out (or in). As mentioned, fast synaptic transmission and spike generation (and hence tetrodotoxin-sensitive sodium channels) are not required. The hyperpolarising phase of the oscillation appears to at least in part involve activation of SK-type calcium-activated potassium currents, given the effects of blocking these currents on the oscillation. Other candidate hyperpolarising currents include the A-type potassium current, which is prominent in these cells (55,56), and inwardly-rectified potassium currents (56). Candidates for depolarisation include hyperpolarisation-activated cation currents, calcium currents and TRPC -mediated currents (35,57–59). Additionally, GnRH neurones maintain high chloride levels in adulthood (60,61) and hence chloride efflux could also be a mechanism for the depolarising phase, as in the interstitial cells of Cajal, which serve as pacemakers within the gastrointestinal tract and have a similar period (62).

Given the small percentage of GnRH neurones that exhibit parabolic bursting or pacemaker oscillations, one might question their relevance. Rare cells with unique properties can contribute to the organisation of network activity. For example, GABAergic hub neurones in the hippocampus are rare cells showing high connectivity but are more likely to induce changes in giant depolarising potentials in the local network (63). Oscillating GnRH neurones had the same general appearance as irregular bursters, similar electrophysiological characteristics other than oscillations versus irregular firing, and a similar frequency of ionotropic GABAergic input compared to

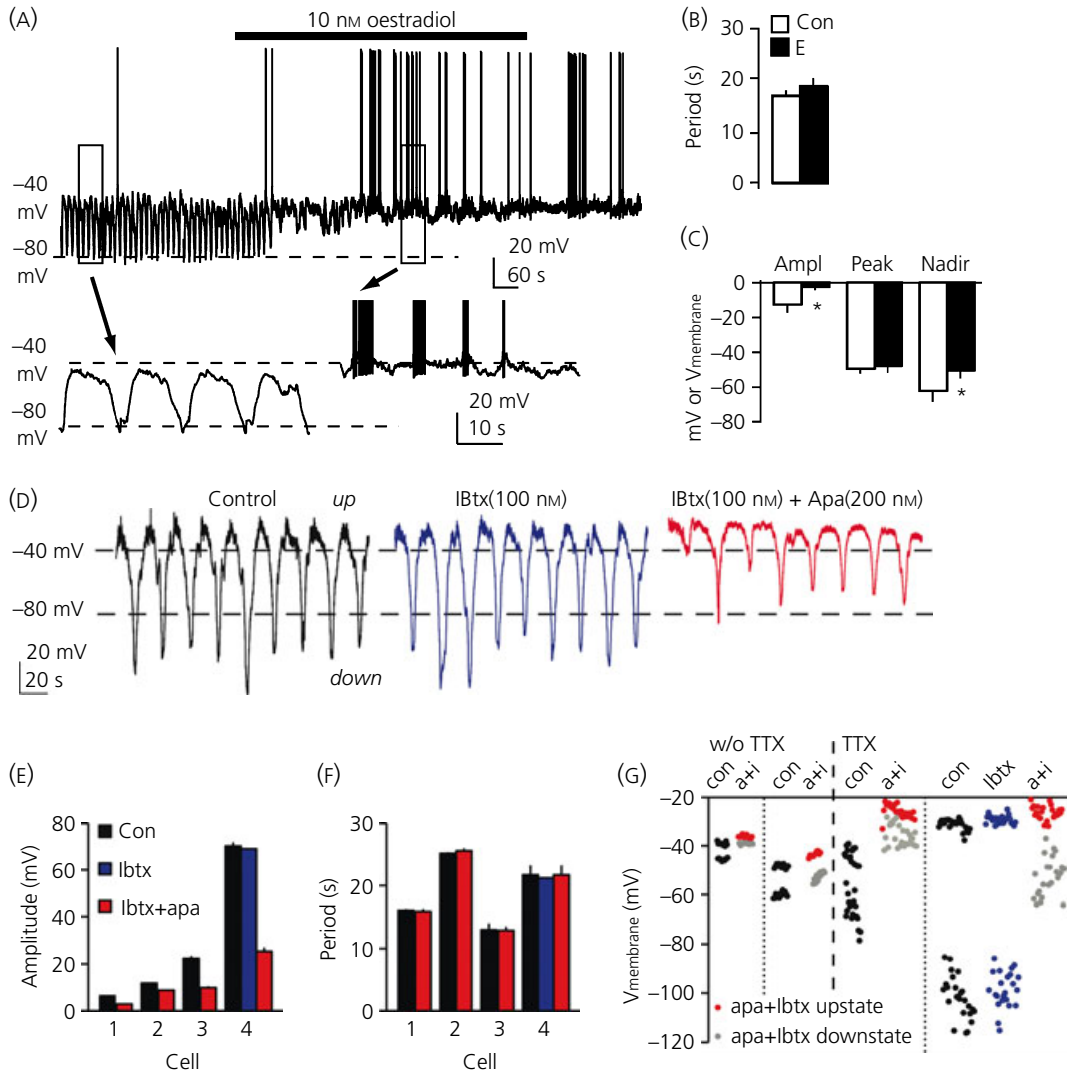

Fig. 7. Oestradiol rapidly alters the oscillation. (A) Gonadotrophin-releasing hormone neurone exhibiting strong subthreshold oscillations begins to spike after the application of oestradiol. Parts of trace in boxes expanded below. (B) Period is not affected by oestradiol. (C) Amplitude is reduced attributable to a change in oscillation nadir. (D) Current-clamp recordings showing that Iberiotoxin (Ibtx) did not alter the oscillation when applied alone but in combination (a + i) with apamin (apa), primarily the downstate of the oscillation is depolarised. (E, F, G) Effect of Iberiotoxin and apamin on oscillation amplitude (E), period (F) and up and down state membrane potential (G). TTX, tetrodotoxin.

previous studies (64,65). On the one hand, the different membrane potential behavior of oscillating cells may point to clear differences in intrinsic properties that could serve a critical role in network function. Mechanistically, the periodic hyperpolarisation could remove inactivation from channels critical for burst firing, and/or could periodically reduce responsiveness to synaptic inputs and thus reduce jitter from ongoing inputs (66). GnRH neurones have high input resistance (9,36,55) and thus may have a high fidelity of excitatory input to spike generation (67). In oscillating cells, spike generation in response to excitatory inputs would undergo a reprieve during a hyperpolarising phase, whereas excitatory input would be more likely to generate spikes during upstates. This would provide an intrinsic gating of the effectiveness of synaptic input. On the other hand, oscillations may be an extreme representation of a periodicity that is more common in GnRH neurones. For example, oestradiol treatment converted subthreshold oscillations into irregular bursts with a similar mean period (Fig. 7).

Perhaps the most interesting putative roles for oscillations are also the most speculative. Oscillations may have a pacemaking role in burst generation and/or in altering the timing of bursts to produce longer-term patterns in overall GnRH neurone firing rate that occur on the timescale of hormone release. With regard to burst firing, a pacemaking role for parabolic oscillations is suggested by their period being tantalisingly close to the interburst interval, as reported in a recent study of GnRH neurone bursting where calcium-activated potassium currents were implicated as being important for generating burst firing in these cells (38). A similar period was also observed in extracellular recordings of physically isolated GnRH neurones (9) and in current-clamp recordings of irregularly bursting GnRH neurones following acute exposure to oestradiol

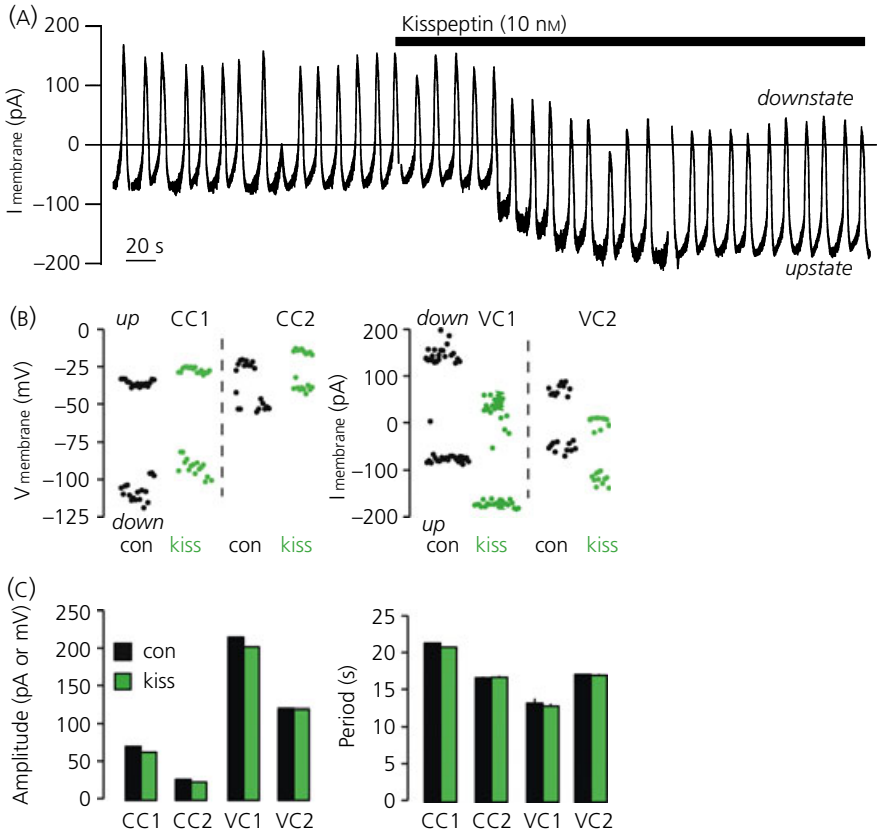

Fig. 8. Kisspeptin shifts the oscillation towards the upstate. (A) Voltage-clamp recording of an oscillating cell before and during kisspeptin treatment showing shift of the entire oscillation towards the upstate (increasingly negative current). (B) Changes in membrane potential (left) and current (right) with kisspeptin. (C) Kisspeptin did not affect oscillation amplitude (left) or period (right). Cell VC2 recorded with 140 mM KCl pipette solution.

(Z. Chu and S. M. Moenter, unpublished observations). With the exception of the current-clamp studies, the methodologies used would not allow the detection of membrane oscillations; however, it is possible that pacemaker oscillations, or at least related mechanisms, underlie these more frequently observable phenomena.

With regard to a role for parabolic oscillations in producing longer-term changes in firing pattern, several observations from the present study are of note. First, we observed cases in which the entire oscillation occurred at subthreshold membrane potentials. This would allow rhythmic activity to be maintained in the absence of spike output from GnRH neurones, perhaps keeping pace during a long interpulse interval or for long durations of quiescence observed in these neurones (28,40,41). Second, in some recordings, action potential spikes occurred only on some oscillation peaks. This would provide a further way to modulate interburst interval. Our data indicate the downstate, rather than the upstate, of oscillations is more variable (Figs 7G and 8B), although even the lesser variability could result in variability of burst generation if the upstate is near the action potential threshold.

Finally, oscillations are modulated by two important inputs to GnRH neurone activity: oestradiol and kisspeptin. In irregularly bursting GnRH neurones in mouse brain slices, both oestradiol and kisspeptin increase firing rate (26,46,57,68,69). Oestradiol rapidly converts oscillations to bursts of similar period, in effect converting an oscillatory signal that was often subthreshold and would not evoke hormone release into a rhythmic signal (bursting) that is optimised for hormone release. Rapid oestradiol action in irregular bursting GnRH neurones appears, in part, mediated by decreasing calcium-activated potassium channels (46). Blocking these channels in GnRH neurones exhibiting parabolic oscillations reduced the amplitude of the oscillation without affecting period. Oestradiol can also rapidly increase both N- and R-type calcium currents (58); the role of these currents in oscillations is not known. By contrast to oestradiol, kisspeptin did not affect oscillation amplitude or period; rather, it translated the oscillation so that the entire oscillation was moved towards the upstate. Kisspeptin is among the strongest known endogenous activators of GnRH neurones (26,68,69). If action potential threshold remains constant, the kisspeptin-induced shift in oscillation range would push more upstates over the threshold for action potential firing and hence increase the overall firing rate. There is a great deal of interest in the role of episodic kisspeptin release (70) as a putative generator of GnRH pulses (71–73).

In summary, although an apparently small number of GnRH neurones exhibit the pacemaker membrane potential oscillations described in the present study, these oscillations could play an important role in the timing of burst activity in the neural population. The ability of two key modulators of GnRH neurones to alter

the parabolic pacemaker oscillations without changing their period suggests a physiological significance in maintaining this rhythm at a relatively constant frequency.

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References

1 Levine JE, Pau KY, Ramirez VD, Jackson GL. Simultaneous measurement of luteinizing hormone-releasing hormone and luteinizing hormone release in unanesthetized, ovariectomized sheep. *Endocrinology* 1982; **111**: 1449–1455.
2 Levine JE, Norman RL, Gliessman PM, Oyama TT, Bangsberg DR, Spies HG. In vivo gonadotropin-releasing hormone release and serum luteinizing hormone measurements in ovariectomized, estrogen-treated rhesus macaques. *Endocrinology* 1985; **117**: 711–721.
3 Clarke IJ, Cummins JT. The temporal relationship between gonadotropin releasing hormone (GnRH) and luteinizing hormone (LH) secretion in ovariectomized ewes. *Endocrinology* 1982; **111**: 1737–1739.
4 Hileman SM, Lubbers LS, Petersen SL, Kuehl DE, Scott CJ, Jackson GL. Influence of testosterone on LHRH release, LHRH mRNA and proopiomelanocortin mRNA in male sheep. *J Neuroendocrinol* 1996; **8**: 113–121.
5 Moenter SM, Brand RM, Midgley AR, Karsch FJ. Dynamics of gonadotropin-releasing hormone release during a pulse. *Endocrinology* 1992; **130**: 503–510.
6 Pitts GR, Nunemaker CS, Moenter SM. Cycles of transcription and translation do not comprise the gonadotropin-releasing hormone pulse generator in GT1 cells. *Endocrinology* 2001; **142**: 1858–1864.
7 Martinez de la Escalera G, Choi AL, Weiner RI. Generation and synchronization of gonadotropin-releasing hormone (GnRH) pulses: intrinsic properties of the GT1-1 GnRH neuronal cell line. *Proc Natl Acad Sci USA* 1992; **89**: 1852–1855.
8 Krsmanovic LZ, Stojilkovic SS, Merelli F, Dufour SM, Virmani MA, Catt KJ. Calcium signaling and episodic secretion of gonadotropin-releasing hormone in hypothalamic neurons. *Proc Natl Acad Sci USA* 1992; **89**: 8462–8466.
9 Kuehl-Kovarik MC, Pouliot WA, Halterman GL, Handa RJ, Dudek FE, Partin KM. Episodic bursting activity and response to excitatory amino acids in acutely dissociated gonadotropin-releasing hormone neurons genetically targeted with green fluorescent protein. *J Neurosci* 2002; **22**: 2313–2322.
10 Nunemaker CS, Straume M, DeFazio RA, Moenter SM. Gonadotropin-releasing hormone neurons generate interacting rhythms in multiple time domains. *Endocrinology* 2003; **144**: 823–831.
11 Terasawa E, Schanhofer WK, Keen KL, Luchansky L. Intracellular Ca(2+) oscillations in luteinizing hormone-releasing hormone neurons derived from the embryonic olfactory placode of the rhesus monkey. *J Neurosci* 1999; **19**: 5898–5909.
12 Jasoni CL, Todman MG, Strumia MM, Herbison AE. Cell type-specific expression of a genetically encoded calcium indicator reveals intrinsic calcium oscillations in adult gonadotropin-releasing hormone neurons. *J Neurosci* 2007; **27**: 860–867.
13 Karsch FJ, Malpaux B, Wayne NL, Robinson JE. Characteristics of the melatonin signal that provide the photoperiodic code for timing seasonal reproduction in the ewe. *Reprod Nutr Dev* 1988; **28**: 459–472.
14 Moenter SM, Caraty A, Locatelli A, Karsch FJ. Pattern of gonadotropin-releasing hormone (GnRH) secretion leading up to ovulation in the ewe: existence of a preovulatory GnRH surge. *Endocrinology* 1991; **129**: 1175–1182.
15 Kim U, McCormick DA. The functional influence of burst and tonic firing mode on synaptic interactions in the thalamus. *J Neurosci* 1998; **18**: 9500–9516.
16 Dutton A, Dyball RE. Phasic firing enhances vasopressin release from the rat neurohypophysis. *J Physiol* 1979; **290**: 433–440.
17 Abe H, Terasawa E. Firing pattern and rapid modulation of activity by estrogen in primate luteinizing hormone releasing hormone-1 neurons. *Endocrinology* 2005; **146**: 4312–4320.
18 Coombes S, Bressloff PC eds. *Bursting: The Genesis of Rhythm in the Nervous System*. Singapore: World Scientific Publishing Co, Ltd, 2005.
19 Frazier WT, Kandel ER, Kupfermann I, Waziri R, Coggeshall RE. Morphological and functional properties of identified neurons in the abdominal ganglion of *Aplysia californica*. *J Neurophysiol* 1967; **30**: 1288–1351.
20 Junge D, Stephens CL. Cyclic variation of potassium conductance in a burst-generating neurone in *Aplysia*. *J Physiol* 1973; **235**: 155–181.
21 Mathieu PA, Roberge FA. Characteristics of pacemaker oscillations in *Aplysia* neurons. *Can J Physiol Pharmacol* 1971; **49**: 787–795.
22 Bertram R, Butte MJ, Kiemel T, Sherman A. Topological and phenomenological classification of bursting oscillations. *Bull Math Biol* 1995; **57**: 413–439.
23 Plant RE. Bifurcation and resonance in a model for bursting nerve cells. *J Math Biol* 1981; **11**: 15–32.
24 Christian CA, Moenter SM. The neurobiology of preovulatory and estradiol-induced gonadotropin-releasing hormone surges. *Endocr Rev* 2010; **31**: 544–577.
25 Oakley AE, Clifton DK, Steiner RA. Kisspeptin signaling in the brain. *Endocr Rev* 2009; **30**: 713–743.
26 Pielecka-Fortuna J, Chu Z, Moenter SM. Kisspeptin acts directly and indirectly to increase gonadotropin-releasing hormone neuron activity and its effects are modulated by estradiol. *Endocrinology* 2008; **149**: 1979–1986.
27 Suter KJ, Song WJ, Sampson TL, Wuarin JP, Saunders JT, Dudek FE, Moenter SM. Genetic targeting of green fluorescent protein to gonadotropin-releasing hormone neurons: characterization of whole-cell electrophysiological properties and morphology. *Endocrinology* 2000; **141**: 412–419.
28 Nunemaker CS, DeFazio RA, Moenter SM. Estradiol-sensitive afferents modulate long-term episodic firing patterns of GnRH neurons. *Endocrinology* 2002; **143**: 2284–2292.
29 Chu Z, Moenter SM. Endogenous activation of metabotropic glutamate receptors modulates GABAergic transmission to gonadotropin-releasing hormone neurons and alters their firing rate: a possible local feedback circuit. *J Neurosci* 2005; **25**: 5740–5749.
30 Paxinos G, Franklin K. *The Mouse Brain in Stereotaxic Coordinates*, 2nd edn. New York, NY: Academic Press, 2001.
31 Barry PH. JPCalc, a software package for calculating liquid junction potential corrections in patch-clamp, intracellular, epithelial and bilayer measurements and for correcting junction potential measurements. *J Neurosci Meth* 1994; **51**: 107–116.

32 Prossnitz ER, Arterburn JB, Smith HO, Oprea TI, Sklar LA, Hathaway HJ. Estrogen signaling through the transmembrane G protein-coupled receptor GPR30. *Ann Rev Physiol* 2008; **70**: 165–190.

33 Tomaiuolo M, Tabak J, Bertram R. Correlation analysis a tool for comparing relaxation-type models to experimental data. *Methods Enzymol* 2009; **467**: 1–22.

34 Ermentrout B. *Simulating, Analyzing, and Animating Dynamical Systems: A Guide to XPPAUT for Researchers and Students*. Philadelphia, PA: Society for Industrial and Applied Mathematics, 2002.

35 Chu Z, Takagi H, Moenter SM. Hyperpolarization-activated currents in gonadotropin-releasing hormone (GnRH) neurons contribute to intrinsic excitability and are regulated by gonadal steroid feedback. *J Neurosci* 2010; **30**: 13373–13383.

36 Suter KJ, Wuarin JP, Smith BN, Dudek FE, Moenter SM. Whole-cell recordings from preoptic/hypothalamic slices reveal burst firing in gonadotropin-releasing hormone neurons identified with green fluorescent protein in transgenic mice. *Endocrinology* 2000; **141**: 3731–3736.

37 Charles AC, Hales TG. Mechanisms of spontaneous calcium oscillations and action potentials in immortalized hypothalamic (GT1-7) neurons. *J Neurophysiol* 1995; **73**: 56–64.

38 Lee K, Duan W, Sneyd J, Herbison AE. Two slow calcium-activated afterhyperpolarization currents control burst firing dynamics in gonadotropin-releasing hormone neurons. *J Neurosci* 2010; **30**: 6214–6224.

39 Nunemaker CS, DeFazio RA, Moenter SM. A targeted extracellular approach for recording long-term firing patterns of excitable cells: a practical guide. *Biol Proc Online* 2003; **5**: 53–62.

40 Pielecka J, Moenter SM. Effect of steroid milieu on gonadotropin-releasing hormone-1 neuron firing pattern and luteinizing hormone levels in male mice. *Biol Reprod* 2006; **74**: 931–937.

41 Pielecka J, Quaynor SD, Moenter SM. Androgens increase gonadotropin-releasing hormone neuron firing activity in females and interfere with progesterone negative feedback. *Endocrinology* 2006; **147**: 1474–1479.

42 Rinzel J, Lee YS. Dissection of a model for neuronal parabolic bursting. *J Math Biol* 1987; **25**: 653–675.

43 Bertram R. Reduced-system analysis of the effects of serotonin on a molluscan burster neuron. *Biol Cybern* 1994; **70**: 359–368.

44 Lyons DJ, Horjales-Araujo E, Broberger C. Synchronized network oscillations in rat tuberoinfundibular dopamine neurons: switch to tonic discharge by thyrotropin-releasing hormone. *Neuron* 2010; **65**: 217–229.

45 Campbell RE, Ducret E, Porteous R, Liu X, Herde MK, Wellerhaus K, Sonntag S, Willecke K, Herbison AE. Gap junctions between neuronal inputs but not gonadotropin-releasing hormone neurons control estrous cycles in the mouse. *Endocrinology* 2011; **152**: 2290–2301.

46 Chu Z, Andrade J, Shupnik MA, Moenter SM. Differential regulation of gonadotropin-releasing hormone neuron activity and membrane properties by acutely applied estradiol: dependence on dose and estrogen receptor subtype. *J Neurosci* 2009; **29**: 5616–5627.

47 Plant RE, Kim M. On the mechanism underlying bursting in the *Aplysia* abdominal ganglion R15 cell. *Math Biosci* 1975; **26**: 357–375.

48 Baer SM, Rinzel J, Carrillo H. Analysis of an autonomous phase model for neuronal parabolic bursting. *J Math Biol* 1995; **33**: 309–333.

49 Canolty RT, Edwards E, Dalal SS, Soltani M, Nagarajan SS, Kirsch HE, Berger MS, Barbaro NM, Knight RT. High gamma power is phase-locked to theta oscillations in human neocortex. *Science* 2006; **313**: 1626–1628.

50 Chapman CA, Lacaille J-C. Intrinsic theta-frequency membrane potential oscillations in hippocampal CA1 interneurons of stratum lacunosum-moleculare. *J Neurophysiol* 1999; **81**: 1296–1307.

51 Steriade M, Contreras D, Curro Dossi R, Nunez A. The slow (< 1 Hz) oscillation in reticular thalamic and thalamocortical neurons: scenario of sleep rhythm generation in interacting thalamic and neocortical networks. *J Neurosci* 1993; **13**: 3284–3299.

52 Steriade M, Amzica F, Nunez A. Cholinergic and noradrenergic modulation of the slow (approximately 0.3 Hz) oscillation in neocortical cells. *J Neurophysiol* 1993; **70**: 1385–1400.

53 Cowan RL, Wilson CJ. Spontaneous firing patterns and axonal projections of single corticostriatal neurons in the rat medial agranular cortex. *J Neurophysiol* 1994; **71**: 17–32.

54 Lampl I, Reichova I, Ferster D. Synchronous membrane potential fluctuations in neurons of the cat visual cortex. *Neuron* 1999; **22**: 361–374.

55 DeFazio RA, Moenter SM. Estradiol feedback alters potassium currents and firing properties of gonadotropin-releasing hormone neurons. *Mol Endocrinol* 2002; **16**: 2255–2265.

56 Zhang C, Bosch MA, Levine JE, Ronnekleiv OK, Kelly MJ. Gonadotropin-releasing hormone neurons express K(ATP) channels that are regulated by estrogen and responsive to glucose and metabolic inhibition. *J Neurosci* 2007; **27**: 10153–10164.

57 Zhang C, Roepke TA, Kelly MJ, Ronnekleiv OK. Kisspeptin depolarizes gonadotropin-releasing hormone neurons through activation of TRPC-like cationic channels. *J Neurosci* 2008; **28**: 4423–4434.

58 Sun J, Chu Z, Moenter SM. Diurnal in vivo and rapid in vitro effects of estradiol on voltage-gated calcium channels in gonadotropin-releasing hormone neurons. *J Neurosci* 2010; **30**: 3912–3923.

59 Sun J, Moenter SM. Progesterone inhibits and androgen potentiates voltage-gated calcium currents in gonadotropin-releasing hormone (GnRH) neurons. *Endocrinology* 2010; **151**: 5349–5358.

60 DeFazio RA, Heger S, Ojeda SR, Moenter SM. Activation of A-type {gamma}-aminobutyric acid receptors excites gonadotropin-releasing hormone neurons. *Mol Endocrinol* 2002; **16**: 2872–2891.

61 Herbison AE, Moenter SM. Depolarising and hyperpolarising actions of GABAA receptor activation on GnRH neurons: towards an emerging consensus. *J Neuroendocrinol* 2011; **23**: 557–569.

62 McHale N, Hollywood M, Sergeant G, Thornbury K. Origin of spontaneous rhythmicity in smooth muscle. *J Physiol* 2006; **570**: 23–28.

63 Bonifazi P, Goldin M, Picardo MA, Jorquera I, Cattani A, Bianconi G, Represa A, Ben-Ari Y, Cossart R. GABAergic hub neurons orchestrate synchrony in developing hippocampal networks. *Science* 2009; **326**: 1419–1424.

64 Sullivan SD, DeFazio RA, Moenter SM. Metabolic regulation of fertility through presynaptic and postsynaptic signaling to gonadotropin-releasing hormone neurons. *J Neurosci* 2003; **23**: 8578–8585.

65 Christian CA, Moenter SM. Estradiol induces diurnal shifts in GABA transmission to gonadotropin-releasing hormone neurons to provide a neural signal for ovulation. *J Neurosci* 2007; **27**: 1913–1921.

66 Schaefer AT, Angelo K, Spors H, Margrie TW. Neuronal oscillations enhance stimulus discrimination by ensuring action potential precision. *PLoS Biol* 2006; **4**: e163.

67 Chadderton P, Margrie TW, Hausser M. Integration of quanta in cerebellar granule cells during sensory processing. *Nature* 2004; **428**: 856–860.

68 Han S-K, Gottsch ML, Lee KJ, Popa SM, Smith JT, Jakawich SK, Clifton DK, Steiner RA, Herbison AE. Activation of gonadotropin-releasing hormone neurons by kisspeptin as a neuroendocrine switch for the onset of puberty. *J Neurosci* 2005; **25**: 11349–11356.

69 Dumalska I, Wu M, Morozova E, Liu R, van den Pol A, Alreja M. Excitatory effects of the puberty-initiating peptide kisspeptin and group I metabotropic glutamate receptor agonists differentiate two distinct subpopulations of gonadotropin-releasing hormone neurons. *J Neurosci* 2008; **28**: 8003–8013.

70 Keen KL, Wegner FH, Bloom SR, Ghatei MA, Terasawa E. An increase in kisspeptin-54 release occurs with the pubertal increase in luteinizing hormone-releasing hormone-1 release in the stalk-median eminence

of female *Rhesus* monkeys in vivo. *Endocrinology* 2008; **149**: 4151–4157.

71 Lehman MN, Coolen LM, Goodman RL. Minireview: kisspeptin/neurokinin B/dynorphin (KNDy) cells of the arcuate nucleus: a central node in the control of gonadotropin-releasing hormone secretion. *Endocrinology* 2010; **151**: 3479–3489.

72 Navarro VM, Castellano JM, McConkey SM, Pineda R, Ruiz-Pino F, Pinilla L, Clifton DK, Tena-Sempere M, Steiner RA. Interactions between kisspeptin and neurokinin B in the control of GnRH secretion in the female rat. *Am J Physiol Endocrinol Metab* 2011; **300**: E202–210.

73 Wakabayashi Y, Nakada T, Murata K, Ohkura S, Mogi K, Navarro VM, Clifton DK, Mori Y, Tsukamura H, Maeda K, Steiner RA, Okamura H. Neurokinin B and dynorphin A in kisspeptin neurons of the arcuate nucleus participate in generation of periodic oscillation of neural activity driving pulsatile gonadotropin-releasing hormone secretion in the goat. *J Neurosci* 2010; **30**: 3124–3132.