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REVIEW

Expanding our horizons: central pattern generation in the context of complex activity sequences

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ABSTRACT

Central pattern generators (CPGs) are central nervous system (CNS) networks that can generate coordinated output in the absence of patterned sensory input. For decades, this concept was applied almost exclusively to simple, innate, rhythmic movements with essentially identical cycles that repeat continually (e.g. respiration) or episodically (e.g. locomotion). But many natural movement sequences are not simple rhythms, as they include different elements in a complex order, and some involve learning. The concepts and experimental approaches of CPG research have also been applied to the neural control of complex movement sequences, such as birdsong, though this is not widely appreciated. Experimental approaches to the investigation of CPG networks, both for simple rhythms and for complex activity sequences, have shown that: (1) brief activation of the CPG elicits a long-lasting naturalistic activity sequence; (2) electrical stimulation of CPG elements alters the timing of subsequent cycles or sequence elements; and (3) warming or cooling CPG elements respectively speeds up or slows down the rhythm or sequence rate. The CPG concept has also been applied to the activity rhythms of populations of mammalian cortical neurons. CPG concepts and methods might further be applied to a variety of fixed action patterns typically used in courtship, rivalry, nest building and prey capture. These complex movements could be generated by CPGs within CPGs ('nested' CPGs). Stereotypical, non-motor, non-rhythmic neuronal activity sequences may also be generated by CPGs. My goal here is to highlight previous applications of the CPG concept to complex but stereotypical activity sequences and to suggest additional possible applications, which might provoke new hypotheses and experiments.

KEY WORDS: CPG, Fixed action pattern, Motor control, Rhythm

Introduction

In 1911, Thomas Graham Brown demonstrated that a cat spinal cord can produce rhythmic output to limb muscles even in the absence of sensory input from the limbs (Brown, 1911) – this rhythmic output underlies walking. In the 1930s, Erich von Holst showed that fish can also generate rhythmic swimming movements after sensory feedback is eliminated (von Holst, 1937, 1973). Both of these findings were largely ignored, however, until Donald M. Wilson similarly demonstrated that the thoracic nerve cord of locusts can generate rhythmic motoneuron output for flying, even in the absence of patterned sensory input (Wilson, 1961). This led Wilson to 'the hypothesis of a built-in central pattern which is not dependent upon peripheral feedback loops for its basic operation'. Thus, the field of central pattern generation was born. In the years following, central pattern generators (CPGs; see Glossary) were demonstrated for a wide array of natural movements, including multiple forms of locomotion, breathing, scratching, calling, chewing, stridulating, digesting and copulating (Arshavsky et al., 2016; Delcomyn, 1980; Marder and Calabrese, 1996), although it was also recognized that sensory feedback modifies these basic movement patterns as needed, especially under rapidly changing environmental conditions. CPGs were found in vertebrates and invertebrates, for both episodic behaviors (like locomotion) and continuous behaviors (like breathing). The physiological mechanisms underlying CPGs were studied at multiple levels of analysis, especially for small invertebrates (Arshavsky et al., 2016; Harris-Warrick, 2010; Marder and Calabrese, 1996; Marder et al., 2014; Selverston, 2010), and further elucidated by computational modeling (Grillner, 2006; Hull et al., 2016; Marder et al., 2014; Selverston, 2010).

Brown, von Holst and Wilson developed the idea of central pattern generation in their studies of rhythmic locomotion but did not restrict its application to rhythmic movements. During subsequent decades, however, the term, 'central pattern generation' was used almost exclusively to describe the control of simple, innate, rhythmic movements in which the identical movement element or set of elements is repeated cyclically, though its cycle period might vary (see Box 1). One may symbolically denote the cyclical repetition of a single movement element, A, as AAAAA…. Cycles that include two, three or more elements, each occurring at its own moment, or phase, of the cycle, may be represented as ABCABCABCABC…, for example. But there is nothing about the basic idea of a CPG – that the central nervous system (CNS) can generate patterned output underlying coordinated movement in the absence of ongoing patterned sensory input – that need limit its application to rhythmic repetition of one cycle. Movement sequences that include different elements in a stereotypical but complex order, such as ABCDEFG… or ABBCDDD…, for example, can be generated by the CNS in the absence of patterned sensory input as well, though this may not be widely appreciated.

Furthermore, the CPG concept need not be restricted to innate behaviors, though that has been its typical application. The CPG concept has also been applied to the control of stereotypical yet complex and non-rhythmic movement sequences, whether innate or learned (Amador et al., 2013; Armstrong and Abarbanel, 2016; Barlow and Estep, 2006; Gracco and Abbs, 1988; Grillner, 1982; Long et al., 2010; Solis and Perkel, 2005; Vu et al., 1994), and even to neural activity sequences that do not directly cause movement (Churchland et al., 2012; Traub et al., 2017; van Dijk and van der Velde, 2015; Yuste et al., 2005). Here, I review a diverse array of such uses of the CPG concept, which I argue are valuable in guiding our thinking and in highlighting experimental approaches that improve our understanding of the generation of complex but stereotypical neural sequences, including learned sequences.

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Journal of Experimental Biology (2019) 222, jeb192054. doi:10.1242/jeb.192054

Glossary

Central pattern generator
A neuronal circuit within the central nervous system that generates patterned output in the absence of continuing patterned sensory input.

Cortical oscillations
Rhythmic electrical signals due to the synchronized activity of many cerebral cortical neurons.

Fictive motor pattern
A pattern of motor neuron activity recorded in the absence of movement.

Fixed action pattern
A natural behavioral sequence that, once begun, is completed, even if its sensory trigger is no longer present.

Ganglia (singular: ganglion)
In invertebrates, separate clusters of neuronal cell bodies (along with synaptic inputs to them) within the central nervous system.

Limb enlargement
In limbed vertebrates, the widening of the spinal cord in spinal cord segments that receive sensory input from and send motor output to a limb.

Pyloric rhythm
One of several centrally generated rhythmic motor patterns that mediate digestion in crustaceans.

Sign stimulus
A specific environmental stimulus that selectively triggers a certain behavior.

Box 1. Central pattern generation through the decades

Statements about central pattern generation have evolved over more than a century and many authors now apply the concept exclusively to rhythmic activity. Below are examples from key articles.

- 'The rhythmic sequence of the act of progression is consequently determined by phasic changes innate in the local centres [in the spinal cord], and these phases are not essentially caused by peripheral stimuli' (Brown, 1911).
- 'if one takes an eel and severs all the peripheral nerves on both sides … so that the head and tail sections are only connected by nervous transmission along the dorsal nerve cord these two ends of the body still swim with the opposing order which they exhibited in the intact fish…. The nervous system is not, in fact, like a lazy donkey which must be struck … every time before it can take a step. Instead it is rather like a temperamental horse which needs the reins just as much as the whip' (von Holst, 1937).
- 'This reduction of [sensory] input did not, however, upset the basic pattern of wing movements including wing twisting and segmental phase differences. This surprising result led to the hypothesis of a built-in central pattern which is not dependent upon peripheral feedback loops for its basic operation, but which is modified by such input' (Wilson, 1961).
- 'the central nervous system does not require feedback from sense organs in order to generate properly sequenced, rhythmic movement during repetitive behaviors such as locomotion' (Delcomyn, 1980).
- 'Rhythmic movements in animals are controlled by neural networks that provide the timing of motoneuron discharge. The central components of these networks are capable of producing rhythmic patterns of activity, although sensory information may be essential for the appropriate response of these networks to behavioral requisites' (Marder and Calabrese, 1996).
- 'CPGs can be defined as functional circuit modules that generate intrinsic patterns of rhythmic activity independent of sensory inputs' (Yuste et al., 2005).
- 'Central pattern generators (CPGs) are defined as central nervous system networks that generate periodic activity in the absence of periodic sensory input' (Golowasch, 2019).

A CPG underlying bird song

A primary candidate for a complex movement sequence that is likely to be CPG generated, and at least partly learned, is birdsong (Brainard and Doupe, 2013; Mooney, 2009). Songs are used primarily by male songbirds to attract mates and deter rivals. Many species sing a complex yet stereotypical sequence of distinct syllables in a particular order, called a motif. For example, a zebra finch motif involves a syllable sequence such as AABCDEFG. In many species, these songs must be learned. Juvenile birds must hear and memorize the song of a tutor (often the father) and later practice their own song, with auditory feedback (Konishi, 1965), to gradually converge on their own version. Birds that never hear a tutor song or do not hear themselves sing never sing a normal song, demonstrating the importance of learning.

Once a male songbird has learned to sing, deafening it does not immediately prevent it from singing a normal song. Konishi suggested that this was either because the song was CPG generated or because non-auditory sensory feedback, such as proprioceptive feedback, is sufficient to generate the motif (Konishi, 1965, 1985, 2010). Initially, Konishi regarded the CPG hypothesis as 'unlikely' (Konishi, 1965); however, Nottebohm subsequently found that song could also continue without proprioceptive feedback from the syrinx (Nottebohm, 1967). Despite this finding, Konishi later argued that the use of 'CPG' should be restricted to innate bird calls and not used for learned motifs, because he regarded CPGs as 'fixed' (Konishi, 2010). I would argue, however, that birdsong is CPG generated if the appropriate sequence of motor outputs can occur without ongoing sensory feedback, regardless of whether sensory feedback is required for its development or maintenance. CPGs often change during development (Marder and Rehm, 2005) and are subject to neuromodulation and plasticity even in adulthood (Marder, 2012; Marder et al., 2014), over time scales of seconds to years.

A forebrain nucleus, HVC, was identified as an important part of the singing pathway (Nottebohm et al., 1976) and became a focus of research. Hahnloser et al. (2002), using single-neuron recordings of HVC neurons that project axons to the robust nucleus of the arcopallium (RA), a premotor structure, demonstrated that each RA-projecting HVC neuron fires action potentials during a remarkably brief and stereotypical period of about 5–10 ms within the 1 s-long motif of a zebra finch. RA-projecting HVC neurons depolarize suddenly for 5–10 ms, each at a certain moment during the song motif, consistent with an HVC CPG comprising a chain of neurons linked by synaptic connections (Long et al., 2010). HVC has been modeled as a CPG or network of CPGs (Amador et al., 2013; Armstrong and Abarbanel, 2016). For these reasons, I will use birdsong as my primary example of a complex movement sequence that is non-rhythmic, at least partly learned and likely to be CPG generated; thinking of birdsong in this way may inspire experimental approaches for the investigation of other complex movement sequences.

Methods used in the study of CPGs

Although the 'gold standard' of demonstrating a CPG is that the CNS is shown to generate appropriately patterned output in the absence of ongoing patterned sensory input, certain other experimental approaches have often been applied in studies of the rhythmic, cyclical movements that are conventionally defined as CPG generated. These experimental approaches can provide important information about CPG components and function, as well as circumstantial evidence for a CPG in systems for which the gold-standard experiment is not feasible, for one reason or another. Experimental results suggesting the existence of a CPG include: (1) for episodic movement sequences, a brief sensory stimulus triggers

a long-lasting movement sequence (or its neural correlate), typically for several seconds, in the absence of continuing sensory neuron activity; (2) depolarization or hyperpolarization of a key CNS neuron(s) resets the timing of an ongoing movement sequence well beyond the movement element during which stimulation occurred; and (3) warming or cooling a contributing CNS region speeds up or slows down the entire sequence, without altering the relative timing of its elements. In addition to providing circumstantial evidence that a movement sequence is essentially CPG generated, these three experimental approaches may help to narrow down the location and composition of CPG components, which is especially useful when it is not practical to study the generation of the movements in the absence of all sensory feedback. These three experimental approaches have also been applied fruitfully to some more complex movement sequences, including some that are at least partly learned, such as birdsong. In the following sections, I will consider some examples of each of these three experimental approaches, first for simple rhythms and then for more complex movement sequences.

Brief sensory stimulation triggers long-lasting movement sequences

Experimental work has shown that, for CPG-generated patterns, a brief sensory stimulus triggers a long-lasting movement sequence or motor pattern (Fig. 1). For example, tail pinch for ∼1 s triggers ∼30 s of sinusoidal forward swimming in a medicinal leech *in vivo*, and similarly brief sensory nerve electrical stimulation elicits ∼30 s of the swimming motor pattern in the leech nerve cord *in vitro* (Fig. 1A) (Mullins et al., 2011; Stent et al., 1978). The fact that this occurs in the absence of actual movement, and therefore in the absence of movement-related sensory feedback, demonstrates that a CPG mediates the continuing swim motor output following brief sensory input. Likewise, the touch of a starfish's tube feet to the mollusk *Tritonia* triggers an escape swimming movement consisting of alternating ventral and dorsal body flexions and lasting ∼1 min *in vivo*, and pedal sensory nerve stimulation triggers ∼1 min of the rhythmic swimming motor pattern *in vitro* (Fig. 1B) (Frost and Katz, 1996). Skin stimulation for a fraction of a second triggers many seconds of sinusoidal forward swimming in a moving hatchling *Xenopus* tadpole and several seconds of the rhythmic forward swimming motor pattern in a tadpole immobilized by neuromuscular junction blockade and thus lacking movement-related sensory feedback (Fig. 1D) (Li and Moult, 2012). In each of these examples, the translation of a brief sensory input into a long-lasting locomotor movement sequence by a CPG presumably mediates a sustained escape movement sequence that carries the animal away from a potential predator. But such findings are not limited to locomotor or escape movements. For example, 1 s of sensory stimulation also can trigger more than 10 s of a scratching motor pattern in an immobilized turtle (Fig. 1C) (Juranek and Currie, 2000).

As with these simple, presumably innate, CPG-driven rhythms, brief sensory stimulation can also trigger long-lasting movement sequences that are more complex and, in some cases, at least partly learned. For example, a male songbird often responds to a rival's song (or even playback of its own song) by singing its own motif in response. This response can continue for several seconds following the end of the (auditory) sensory stimulation that provoked it (Fig. 1E) (Prather et al., 2008). Thus, this complex, learned movement sequence continues for several seconds following the end of its sensory trigger, just as for the simple, innate, CPG-driven rhythms described above. Just before the song motif begins, the bird typically sings 'introductory' notes. Recent evidence suggests that, during this introductory period, neural excitation in HVC builds to a threshold that, once exceeded, triggers the motif to begin and to continue until completed (Rajan, 2018) and that this can occur independent of sensory feedback (Rao et al., 2019). Thus, excitation may build up until it activates a singing CPG composed of RA-projecting HVC neurons (Daliparthi et al., 2019).

The continuation of a movement sequence for many seconds following a brief sensory trigger (in this context often called a 'sign stimulus'; see Glossary), typically until an adaptive endpoint, is also part of what early ethologists termed a 'fixed pattern' or 'fixed action pattern' (see Glossary; Ronacher, 2019; Tinbergen, 1951). These ethologists focused on complex natural movements that include distinct elements in a stereotypical sequence, though they assumed that the capacity to produce these sequences is innate. An example of a fixed action pattern is the egg-rolling behavior of the graylag goose (Lorenz and Tinbergen, 1939). When a female goose sees an egg in front of her nest, she moves her neck and bill to roll the egg toward the nest. If the egg is removed midway through this process (i.e. the sensory stimulus is gone), she continues to make the rolling movements until her bill reaches the nest. Thus, the movement sequence continues well beyond the sensory stimulus that triggered it.

Such fixed action patterns have been described for a wide variety of behaviors in an array of vertebrates and invertebrates, especially for movement sequences used in courtship, nest building, aggression and prey capture (Tinbergen, 1951). In each case, the movement sequence continues to a behavioral endpoint even if the triggering sensory stimulus ends. In nearly all such cases, the neural control of the movement sequence is not well understood. This is perhaps unsurprising, because these movement sequences are typically complex; also, there is no obvious way to elicit these movement sequences in an animal that is semi-intact or immobilized, or in an *in vitro* CNS, which would facilitate narrowing down the key CNS region(s) involved.

I suggest, however, that brief artificial activation of the key CNS region may be found to generate long-lasting patterned motor output that mirrors the fixed action pattern, much as seen for simple CPG-driven rhythms. If so, one might conclude that these fixed action patterns are each CPG driven and that the CPG is partly or entirely contained within the CNS region thus activated. In fact, it can be fruitful to search in reduced preparations for motor sequences that appear to be related to fixed action patterns that can be triggered by either a natural sensory stimulus or electrical stimulation of sensory neurons that mimics a sign stimulus, as a means to identify the CPG-like neural circuits that generate this motor output.

For example, a songbird's motif is arguably a fixed action pattern, and some investigations have attempted to trigger it in a reduced preparation. Brief, high-frequency electrical stimulation within *in vitro* zebra finch HVC slices can trigger seconds-long series of repetitive electrical events in HVC with similar timing to the normal song syllables (Solis and Perkel, 2005). In contrast, such responses are usually not seen in RA following similar RA electrical stimulation. It is likely that electrical stimulation of HVC or its input pathways that more closely mimics the neural response to a sign stimulus (such as a rival's song) would trigger a more naturalistic pattern of HVC neural activity. Such experiments should help to reveal the neural mechanisms in HVC that generate the motif.

Although the original concept of a fixed action pattern referred to innate behaviors, this concept may also be useful for learned behavioral sequences like birdsong. A variety of other learned,

Journal of Experimental Biology (2019) 222, jeb192054. doi:10.1242/jeb.192054

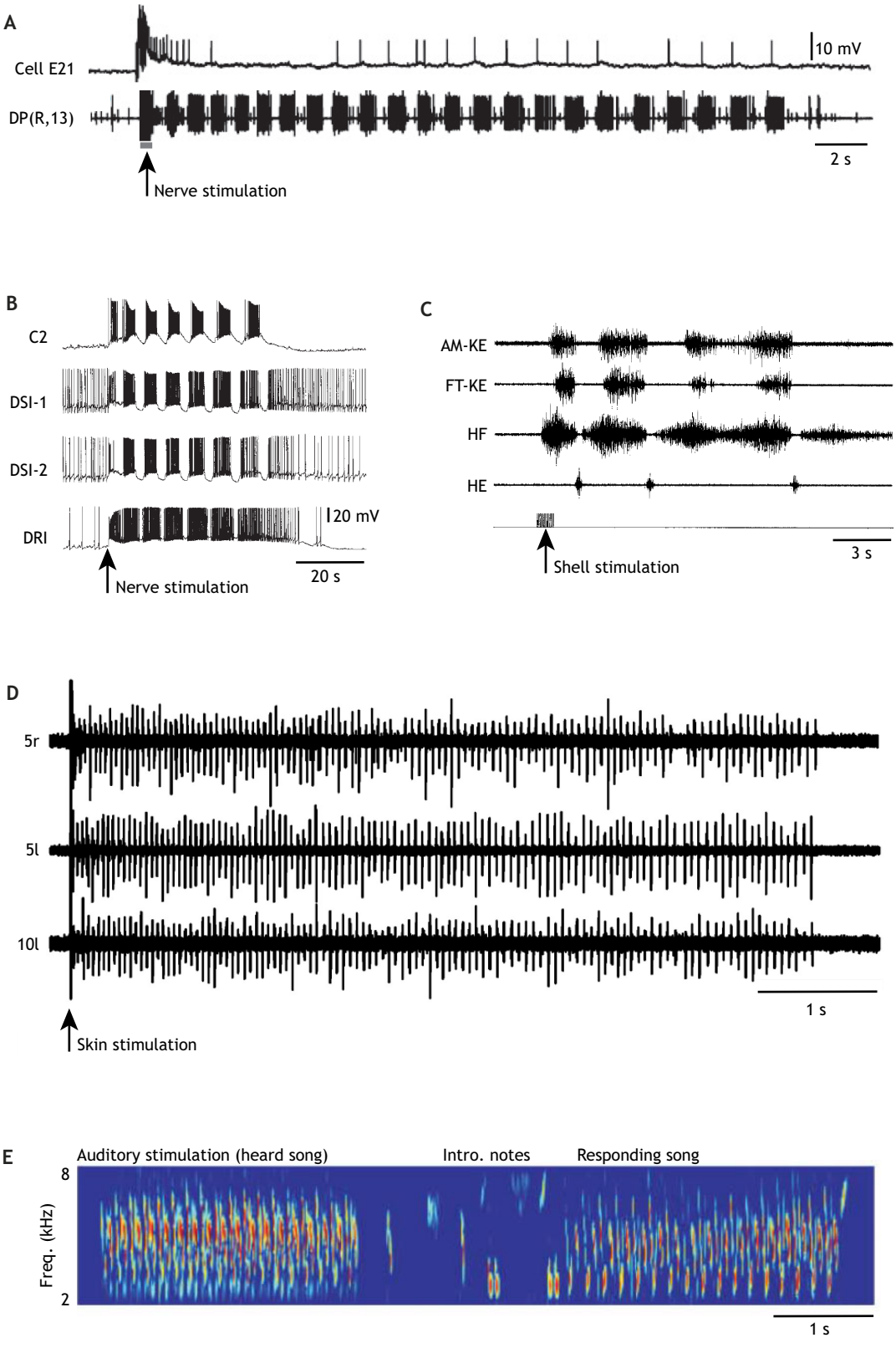

Fig. 1. See next page for legend.

complex, behavioral sequences in an array of species may exhibit similar characteristics, with the entire sequence being completed once triggered. In a variety of vertebrates, many habits may be mediated by basal ganglia-based circuits that are constructed during a learning phase and later triggered by environmental conditions (Graybiel, 2008; Jog et al., 1999). In most cases, these habits are adaptive, improving efficiency and reducing the cognition necessary for daily activity sequences, such as brushing our teeth.

Fig. 1. Examples of brief sensory neuron activation that triggers a long-lasting motor sequence. (A) Brief electrical stimulation of a leech nerve *in vitro* causes a brief burst of action potentials in an intracellularly recorded trigger neuron, cell E21, and a long-lasting series of rhythmic bursts of action potentials in an extracellularly recorded motor nerve [DP(R,13): DP, dorsal-posterior nerve; R, right.] that represents the leech swimming motor pattern. (B) Brief electrical stimulation of a *Tritonia* sensory nerve *in vitro* triggers a long-lasting series of rhythmic bursts of action potentials in several intracellularly recorded neurons that produce the *Tritonia* swimming motor pattern. C2, cerebral cell 2; DSI, dorsal swim interneuron; DRI, dorsal ramp interneuron. (C) Brief electrical stimulation of the shell of an immobilized turtle *in vivo* triggers several rhythmic bursts of action potentials in extracellularly recorded knee extensor (KE: AM, ambiens; FT, femorotibial), hip flexor (HF) and hip extensor (HE) nerves that mediate turtle scratching. (D) Brief electrical stimulation of an immobilized tadpole's skin *in vivo* triggers a long-lasting series of rhythmic action potentials in extracellularly recorded motor neurons in the 5th and 10th spinal cord segments that underlie tadpole swimming. r, right; l, left. (E) Playback of a swamp sparrow's song causes the bird to sing its own song motif for several seconds afterwards. A–E adapted from (A) Mullins et al. (2011), (B) Frost and Katz (1996) (copyright National Academy of Sciences USA), (C) Juranek and Currie (2000), (D) Li and Moult (2012) and (E) Prather et al. (2008).

In other cases, such as Tourette syndrome and obsessive–compulsive behavior, such habits may be maladaptive. One way of looking at the process of habit formation, whether the habit is adaptive or maladaptive, is that new CPGs are constructed during a learning phase, perhaps within the basal ganglia, and are later evoked by particular sensory cues.

Electrical stimulation of control elements can reset the timing of a movement sequence

A second experimental approach has traditionally been used to demonstrate that individual neural elements (which, in invertebrates, are typically individually identifiable neurons) are members of a CPG. During an ongoing CPG-driven rhythmic motor pattern, a neuron is briefly depolarized or hyperpolarized. Momentary disruption of the motor pattern would be expected if this manipulation were applied to any neuron that contributes in some way to the motor output. But if a neuron is a member of the CPG, then this stimulation may also alter the timing of subsequent cycles of the rhythm in a lasting manner, causing these later cycles to begin consistently earlier or later than before the stimulation. In other words, stimulation of a CPG element 'resets the clock'. This is evidence that the stimulated element is part of the CPG clock or, at least, has access to the clock.

Let us consider some invertebrate and vertebrate examples of stimulation that resets simple rhythms. When a leech neuron named cell 28 is intracellularly depolarized for ∼1 s during an ongoing swim motor pattern, the next swim cycle, as monitored in extracellular nerve recordings, is delayed until after stimulation ends. More importantly, each subsequent cycle also begins later than it would have (Fig. 2A) (Stent et al., 1978). Intracellular hyperpolarization of a cell 208 neuron (there is one per ganglion; see Glossary) also delays subsequent swim cycles (Fig. 2B) (Weeks, 1982). Thus, in both cases, the leech swim clock has been reset, demonstrating that cells 28 and 208 are members of the leech swim CPG.

A second example comes from cricket stridulation (chirping), which is produced by rhythmic movements of one front wing against the other. The chirping motor pattern can be produced without actual wing movements by pharmacological activation of a specific brain region, which in turn activates a thoracic and abdominal chirping CPG. Intracellular depolarization of an abdominal ganglion neuron named A3-AO during the silent period between fictive chirps (fictive motor pattern; see Glossary) triggers an early next chirp, and each subsequent chirp is also earlier (Fig. 2C) (Schoneich and Hedwig, 2012). This resetting of the chirp rhythm demonstrates that cell A3-AO is a member of the CPG.

Examples of resetting of CPG-driven rhythms usually come from invertebrates, for a good reason: invertebrates have much smaller nervous systems and CPGs than most vertebrates. Even for simple rhythms, depolarization or hyperpolarization of a single neuron in a typical vertebrate CPG is unlikely to reset the rhythm, because the stimulated neuron comprises a small fraction of the entire CPG (Marder and Calabrese, 1996). The effect of single-neuron stimulation is thus massively diluted by the activity of unstimulated CPG neurons, and there may be no detectable effect on the motor rhythm. If, however, an entire set of vertebrate neurons of one type can be simultaneously stimulated, then it may be possible to reset an ongoing rhythm and thus demonstrate that neurons of this type are members of the corresponding CPG.

Using optogenetics, experiments of this sort have been performed in rodents, and the results suggest that one type of brainstem neuron is part of the mammalian breathing inspiratory CPG, previously localized to a medullary structure called the pre-Bötzinger complex (PBC). Optogenetic depolarization of rat PBC neurons as a group for 200 ms caused the next breath to begin immediately following stimulation, no matter when the previous breath had occurred (Alsahafi et al., 2015). Moreover, the subsequent breath also occurred on the new schedule, regardless of previous timing, demonstrating resetting (Fig. 2D). This was perhaps not surprising, given that the inspiratory CPG had previously been localized to the PBC. Later, however, optogenetic stimulation limited to PBC neurons expressing the transcription factor Dbx1 in neonatal mice also induced another breath immediately and shifted the timing of subsequent breaths to the new schedule (Fig. 2E,F) (Cui et al., 2016; Vann et al., 2018). Although these experiments were performed on rodents that were actually breathing, it seems likely that future studies will demonstrate such resetting by stimulation of Dbx1+ PBC neurons during fictive or *in vitro* inspiratory rhythms, which would suggest via rhythm reset that neurons of this type are constituents of the mammalian inspiratory CPG.

The examples outlined above relate to rhythmic movement patterns, but what about more complex movement sequences? At first, it might seem that resetting of such sequences could not even be defined, because without a simple rhythm, there is no simple clock. But even for movement sequences comprising distinct elements of different durations, each movement element typically begins at a stereotypical moment, has a stereotypical duration and occurs in a stereotypical order, with the duration of the entire movement sequence also consistent. In such cases, stimulation of CPG elements might cause an observable reset of the timing of the entire sequence. For example, the movement sequence might suddenly stop and begin anew from the start.

Evidence of resetting of a complex movement sequence has been obtained for birdsong (Vu et al., 1994). Normally, the entire motif is repeated with consistent syllable order, syllable timing and motif duration. But 50 ms extracellular stimulation within HVC during an ongoing song can stop the song immediately and cause the motif to restart from the beginning, then continue with the normal syllable order and timing and motif duration (Fig. 2G). In contrast, similar stimulation in a downstream brain region, RA, alters the current syllable without affecting the onset timing or duration of subsequent syllables (Fig. 2H). These findings suggest that HVC contains neurons that are members of a singing CPG, while RA does not.

Journal of Experimental Biology (2019) 222, jeb192054. doi:10.1242/jeb.192054

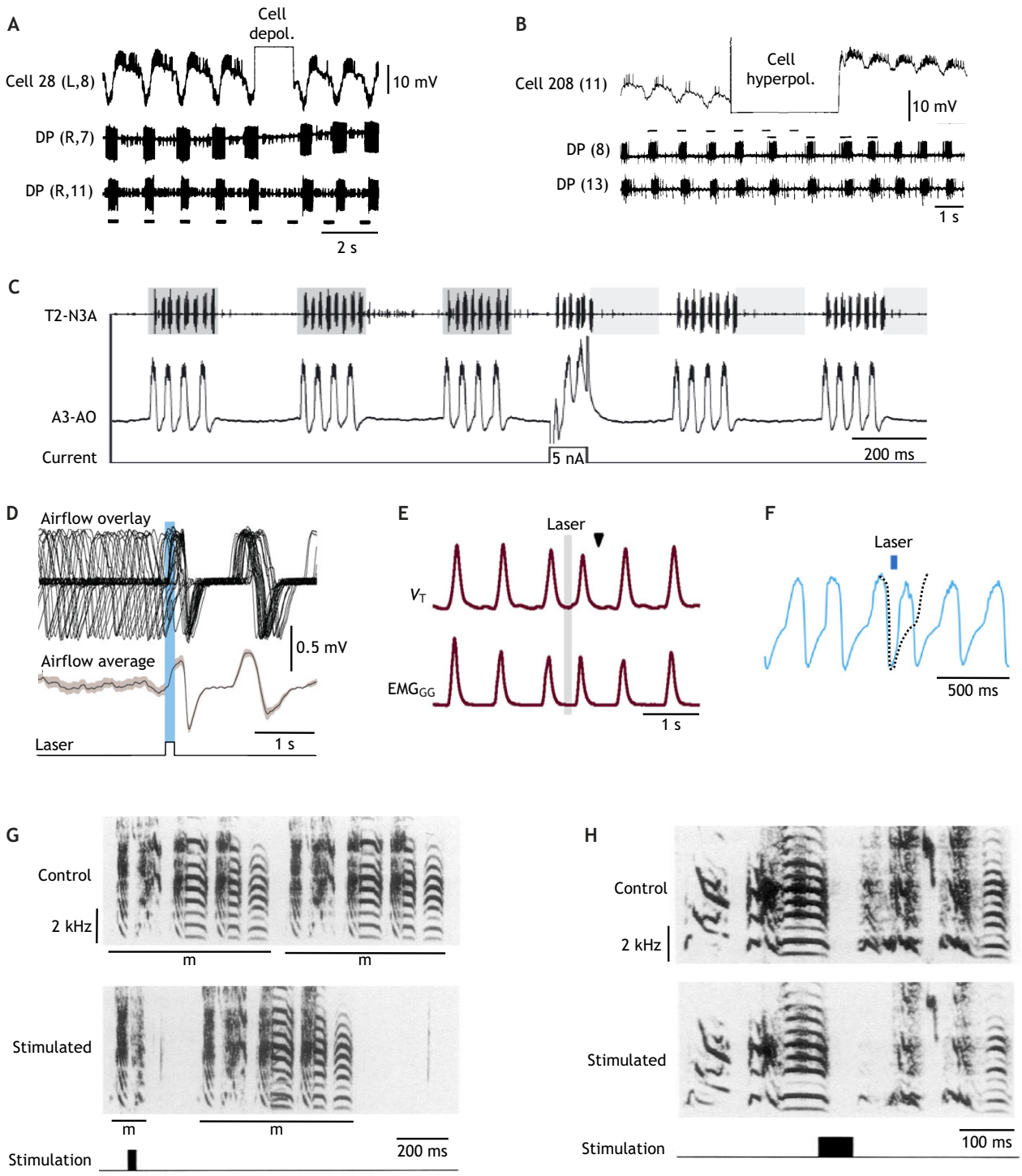

Fig. 2. Examples of brief electrical stimulation that resets a motor sequence. (A,B) Intracellular depolarization of a cell 28 (A) and hyperpolarization of a cell 208 (B) during *in vitro* leech swimming motor patterns each resets the timing of swim-related bursts in extracellularly recorded motor nerves (DP, dorsal-posterior nerve); the original swim burst timing is indicated by horizontal bars in A; numbers in parentheses indicate the ganglion recorded from. R, right; L, left. (C) The timing of fictive cricket chirping, monitored via an extracellularly recorded mesothoracic wing motor nerve (T2-N3A), is reset by depolarization of the intracellularly recorded CPG abdominal ascending opener-interneuron (A3-AO); the original chirp timing is indicated by gray rectangles. (D) Optical activation (blue bar) of light-sensitive ion channels expressed in rat medullary pre-Bötzinger complex (PBC) neurons *in vivo* triggers an immediate breath and resets the timing of the subsequent breath, shown for multiple, superimposed trials. (E,F) Optical activation (gray bar in E and blue bar in F) of Dbx1+ PBC neurons in neonatal mice *in vivo* triggers an immediate breath and resets the timing of subsequent breaths; arrowhead in E and black dotted line in F indicate when the next breath would have occurred if the original timing had been maintained. V_T, tidal volume; EMG_{GG}, genioglossus electromyographic activity. (G,H) *In vivo* zebra finch brain electrical stimulation of the HVC forebrain nucleus (G) ends and restarts the entire song motif (m), whereas electrical stimulation of the robust nucleus of the arcopallium (RA) (H) has only a brief effect and does not alter the timing of subsequent syllables. A–H adapted from (A) Stent et al. (1978), (B) Weeks (1982), (C) Schoneich and Hedwig (2012), (D) Alsahafi et al. (2015), (E) Cui et al. (2016), (F) Vann et al. (2018) and (G,H) Vu et al. (1994) (copyright, Society for Neuroscience).

These experiments were performed in singing animals that have sensory feedback available, so strictly speaking they do not necessarily demonstrate resetting of a CPG-driven sequence, but they provide tantalizing evidence that points toward resetting of an HVC CPG-driven song motif. Birdsong is often considered the best non-human animal analog of human speech, and human speech elements and sequences have also been proposed to be CPG generated (Barlow and Estep, 2006; Grillner, 1982). Perturbation experiments during human speech can have phase-dependent effects (Gracco and Abbs, 1988), just as for simple, rhythmic CPGs (Burke, 1999; Duysens et al., 2013), consistent with human speech being generated at least partly by CPGs. Cognitive processing of sentences has also been modeled in a CPG-based manner (van Dijk and van der Velde, 2015).

Warming or cooling can speed up or slow down the entire movement sequence

A third experimental approach that can indicate which neural elements are part of a CPG is warming or cooling of CNS regions. Warming generally speeds up operations of cells and their constituent proteins, whereas cooling generally slows down cellular functions. For CPGs that drive simple rhythms, warming the CPG generally speeds up the rhythm and cooling the CPG slows it down, while preserving the relative timing, or phasing, of motor pattern elements. This approach has been used in the study of some invertebrate and vertebrate CPGs (Fee and Long, 2011), and I discuss some relevant examples below.

Selective cooling of cat spinal cord segments was used to determine which segments are most important for control of the scratching rhythm. Cooling one side of the second lumbar segment slows down the fictive rhythm while preserving the phasing of rhythmically active motor nerves (Fig. 3A) (Deliagina et al., 1983). This is not true for cooling of more caudal spinal segments. This suggests that key constituents of the scratching CPG are located within the rostral segments of the spinal cord hind limb enlargement (see Glossary), consistent with other kinds of evidence, including the results of lesion experiments (Deliagina et al., 1983; Mortin and Stein, 1989).

In crickets, warming the thorax speeds up an aggressive chirping rhythm, while warming the head does not (Fig. 3B) (Pires and Hoy, 1992). This suggests that key elements of this chirping CPG are contained within the thoracic ganglia, but not the head ganglia. These experiments were performed in animals that were actually singing, so sensory feedback was available, but subsequent experiments showed that cricket courtship chirping CPG neurons are in the thoracic (and abdominal) ganglia (Schoneich and Hedwig, 2012), as suggested by the warming experiments.

The crustacean stomatogastric nervous system generates perhaps the most thoroughly studied simple CPG rhythms, including the pyloric rhythm (see Glossary). Warming or cooling the stomatogastric ganglion *in vitro* dramatically speeds up or slows down the rhythm, respectively, while leaving the phase relationships between neurons unchanged (Fig. 3C) (Tang et al., 2010). Although it was already clear prior to these experiments that the stomatogastric ganglion contains pyloric CPG neurons, these experiments demonstrate the reliability of warming or cooling of CPG elements to alter sequence rate while preserving relative timing.

Male *Xenopus* frogs attract females using an advertisement call that is more complex than the simple rhythms typically studied in CPG research. It comprises an introductory phase, a fast trill (~60 Hz) and a slow trill (~30 Hz), with a slower 'envelope' of fast and slow trill onsets and offsets (~1 Hz). Then, the entire sound sequence repeats with relatively consistent timing. Because there are multiple rhythms within the call, we might regard it as a complex motor sequence, perhaps intermediate in complexity between the simpler rhythms described above and the more complex movement sequences of birdsong and fixed action patterns generally.

The *Xenopus* advertisement call motor pattern can be evoked in the brainstem *in vitro* by application of serotonin, suggesting that this region of the CNS contains a CPG for advertisement calls (Rhodes et al., 2007). Cooling the whole brainstem *in vitro*, or just one medullary nucleus – the dorsal tegmental area of the medulla (DTAM) – slows both the fast and the slow trill (Yamaguchi et al., 2008). If DTAM is cut off from other brainstem regions, it can still generate the ~1 Hz envelope of fast trills. Warming or cooling the bathing solution speeds up and slows down, respectively, the rate of the fast trill envelope in DTAM when it is isolated from the motor nucleus *in vitro* (Fig. 3D, right); this is also observed following warming or cooling of the whole brainstem *in vitro* (Fig. 3D, left) (Zornik et al., 2010). Thus, one might think of the advertisement call CPG as two faster CPGs (for fast- and slow-trill clicks) governed by a slower CPG that determines the onset and offset of each faster rhythm; cooling one of these CPGs may selectively slow just one of these rhythms.

One way to think of this organization is that a number of simpler and faster CPGs are functionally (though not necessarily anatomically) nested within an overarching CPG (Fig. 4). In other words, each component CPG generates a relatively fast rhythmic motor pattern on its own whenever triggered. An additional CPG exists at a higher hierarchical level and incorporates the faster CPGs, rather than individual neurons, as its components. This overarching CPG has synaptic connections that trigger each component CPG to generate its own rhythm at a particular moment within the slower sequence. This idea has much in common with Grillner's unit burst generator concept: the idea that bursts of neural activity triggering flexion or extension of a single joint can be flexibly coupled by different higher-order CPGs to generate appropriate intralimb coordination (Grillner, 1981). Flexible interactions of unit CPGs can also account for coordination of cyclical activity across spinal cord segments during undulatory swimming and for interlimb coordination in limbed locomotion (Grillner, 2006). For the frog advertisement call, each component of the overarching CPG appears to generate a different, rhythmic sequence of bursts, rather than a single burst, and thus may be at a still higher organizational level.

Perhaps it would be useful to think of CPGs within CPGs within CPGs. The component CPGs could be either anatomically intermingled with one another within one CNS region or anatomically separate but linked through long-distance synaptic connections of the overarching CPG. For example, vocalizing and breathing in songbirds are coordinated during singing (Schmidt and Goller, 2016; Schmidt and Wild, 2014; Wild et al., 1998), as during human speech (Brainard and Doupe, 2013). Although this coordination may at least partly be due to sensory feedback (Schmidt and Goller, 2016; Suthers et al., 2002), there might also be an overarching CPG that links the two. Similarly, individual CPGs for locomotion and breathing in mammals may be coordinated via synaptic connections that increase respiratory rate at the start of locomotion (before chemosensory feedback changes) and decrease it later (Le Gal et al., 2014, 2016), perhaps constituting a CPG at a higher hierarchical level and a slower time scale than the component CPGs.

Warming or cooling experiments can also be useful when thinking about motor sequences that are more complex than frog calling. Let

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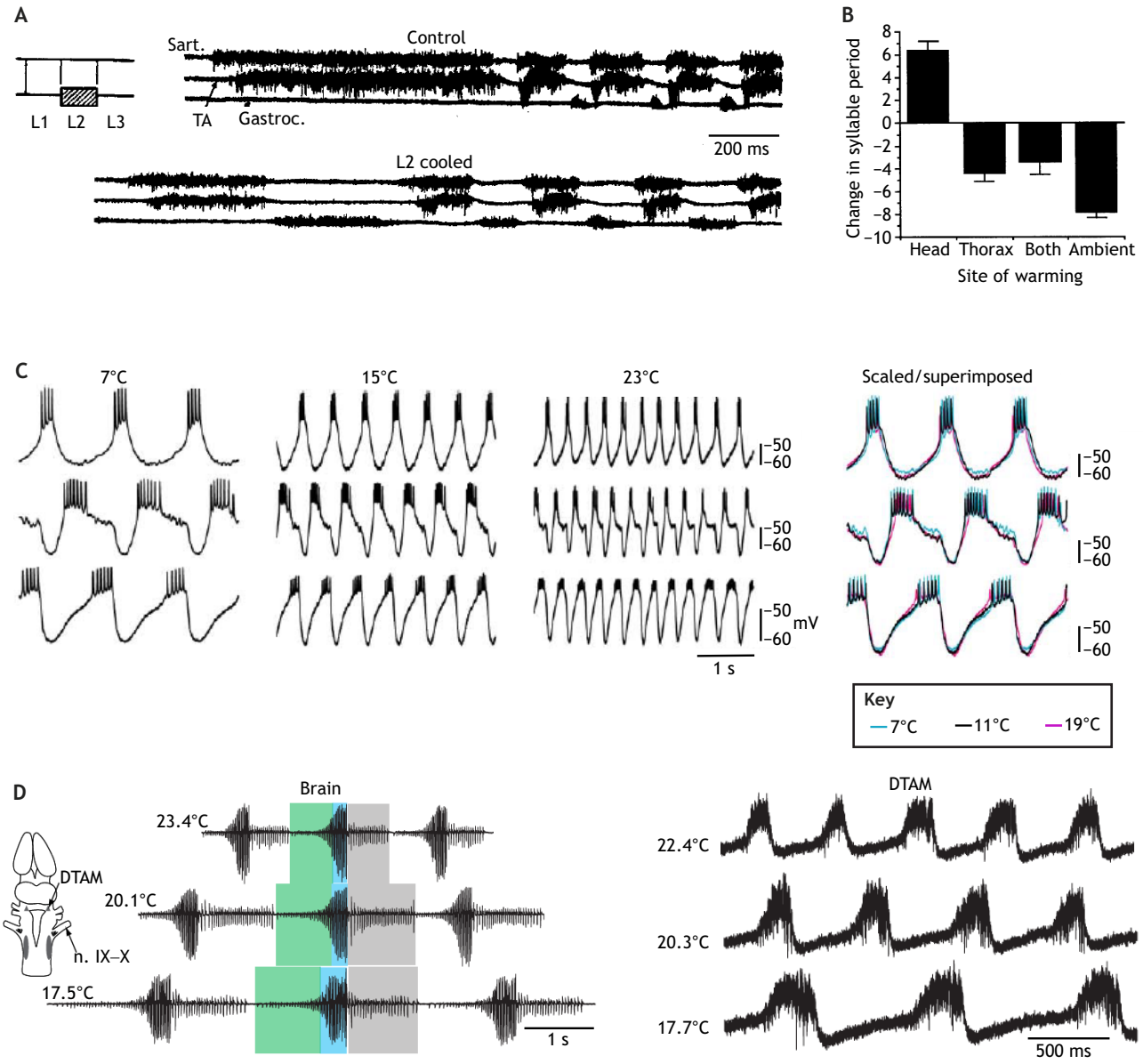

Fig. 3. Examples of central nervous system (CNS) temperature changes that alter motor sequence rate but preserve relative timing. (A) Cooling the second lumbar spinal cord segment on one side (hatched rectangle) slows the rhythm of cat fictive scratching *in vivo* without altering phase relationships between the extracellularly recorded motor nerves. L, lumbar segment; Sart., sartorius nerve; TA, tibialis anterior nerve; Gastroc., gastrocnemius nerve. (B) Warming a cricket's thorax (as well as both its thorax and its head, or the ambient temperature of the room), but not its head alone, decreases the cycle period (i.e. increases the rhythm rate) of cricket aggressive chirping. (C) Rhythmic depolarization and bursts of action potentials in three intracellularly recorded neurons mediating a crab stomatogastric pyloric rhythm *in vitro*, at three different temperatures (left) and with three temperatures superimposed after temporal scaling (right); note that the rhythm rate changes dramatically with temperature but relative timing is maintained. (D) Serotonin-triggered male *Xenopus* fictive advertisement calls *in vitro* monitored via extracellular motor nerve recordings (n. IX–X) in the whole brain (left) and via local field potential recordings in the brainstem dorsal tegmental area of the medulla (DTAM) nucleus cut off from the motor nucleus (right) at three temperatures. Note that temperature change alters the rate but not the relative timing of the activity sequences and that the isolated DTAM could generate the timing of the fast trills in the absence of the other sequence elements. Green, blue and gray boxes on the left indicate introductory notes, fast trill and slow trill, respectively. A–D adapted from (A) Deliagina et al. (1983), (B) Pires and Hoy (1992), (C) Tang et al. (2010) and (D) Zornik et al. (2010).

us again turn to birdsong. Above, I discussed the idea that electrical stimulation of HVC can reset a song motif. Warming or cooling HVC in zebra finches can speed up or slow down singing, respectively, with each syllable occurring at the same relative time as before, but the entire motif being shorter or longer, much as if a record or tape were played faster or slower (Fig. 5A) (Long and Fee, 2008). This can be nicely demonstrated by compressing or stretching the *x*-axis of a control motif sonogram; the results closely match song motif sonograms that are obtained experimentally by warming or cooling HVC, respectively (Fig. 5A, bottom).

Cooling another zebra finch brain nucleus, uvaformis (Uva), which has neuronal projections to HVC, can similarly slow down the entire motif while preserving the relative timing of syllables, even without substantially altering HVC temperature (Fig. 5B) (Hamaguchi et al., 2016). Thus, either Uva and HVC may both contain constituents of a CPG for singing, or cooling Uva may

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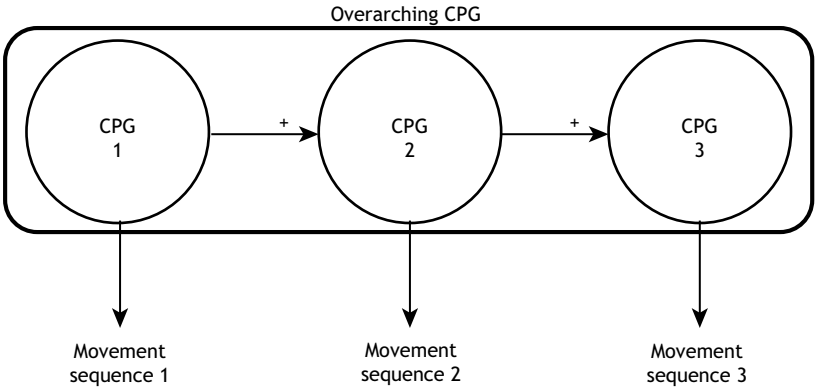

Fig. 4. Schematic diagram illustrating nested CPGs, or CPGs within a CPG. Each movement sequence or activity sequence, whether rhythmic or non-rhythmic, could be generated by its own CPG (CPGs 1–3) and these CPGs could themselves be synaptically connected to one another (+) to form an overarching CPG that triggers an appropriate sequence of rhythms or sequence of activity sequences.

indirectly slow the movement sequence by reducing excitation of HVC. This set of experiments thus highlights a caveat of warming/cooling experiments, namely that it may not be the CPG itself that is manipulated to produce the predicted change, but instead neurons that excite or inhibit the CPG. Perhaps an analogy would be stimulation of the descending brain pathways that trigger locomotion in vertebrates; increased stimulation of these pathways can increase the rate and even alter the form of locomotion, presumably by increasing excitation of spinal cord CPGs (Cabelguen et al., 2003; Ferreira-Pinto et al., 2018; Lennard and Stein, 1977).

Warming or cooling HVC in Bengalese finches, besides altering the motif duration, can alter the number of syllables of one type (Fig. 5C) (Zhang et al., 2017). For example, if the control motif is represented as CAAAAA, it can become CAAAAAAAA with HVC heating and CAAAA with HVC cooling. Thus, heating or cooling putative CPG constituents can alter not just sequence rate but also what has been termed ‘syntax’.

How far can the CPG concept be expanded?

So far, we have seen that three kinds of ‘circumstantial evidence’ applied to simple CPG-driven rhythms may also provide evidence for CPGs producing more complex motor sequences: (1) brief sensory triggers cause long-lasting motor sequences, (2) excitation or inhibition of constituent elements can reset the timing of subsequent movement elements, and (3) warming or cooling of key neural elements can speed up or slow down the sequence while preserving relative timing. Some may argue, however, that the examples of complex motor sequences I have highlighted, such as birdsong, could still be considered a kind of rhythm, albeit on the complex end of the spectrum.

Are there still more complex kinds of movement sequences to which the concept of a CPG and the experimental approaches to studying CPGs could or should be applied? How far can CPG concepts and approaches be usefully expanded?

As alluded to earlier, there are a wide variety of fixed action patterns, some of which include a series of quite different movement elements, each with a distinct duration, such as those that occur during courtship and rivalry displays. In some cases, these movement elements involve different body parts and thus must be controlled by different motoneurons and presumably different interneurons, which might be anatomically separated. If such movement sequences could be shown to be controlled by CPGs, this would probably go beyond the complexity of birdsong.

To take one set of examples, consider multimodal courtship/rivalry displays, which are especially elaborate in male birds (Mitoyen et al., 2019). These displays often combine simultaneous movements of colored feathers, movements of legs and wings, and sounds. These displays are particularly dramatic in birds of paradise (e.g. https://youtu.be/W7QZnwKqopo) and manikins (e.g. https://youtu.be/o42C6ajjqWg). For example, golden-collared manakins jump between samplings and the ground, make ‘helicoptering’ leaps, simultaneously vocalize and make snapping sounds with their wings, and end their display sequence with a series of wing snaps at about 60 Hz (Fuxjager et al., 2018).

These displays necessarily involve coordinated movements of multiple body parts and thus a wide array of motoneurons and interneurons. But all these movements typically are precisely coordinated, maintaining their relative timing as CPG-driven movements do, and are relatively stereotypical within each animal. Could these displays be generated by nested CPGs, in a manner more sophisticated than that which appears to occur for the *Xenopus* advertisement call? Could the rhythmic movement of each body part be generated by its own CPG, with a slower, overarching CPG coordinating the whole set? The individual nested CPGs might be anatomically separated, so long as they have strong connections that link them appropriately; these connections might effectively form the overarching CPG that they nest within. In the example of the golden-collared manikin, the CPG(s) may include androgen-dependent regions of the arcopallium (Fusani et al., 2014).

Finally, let us consider some examples of complex movement sequences that have been triggered by electrical stimulation in the brain. Erich von Holst used *in vivo* electrical stimulation in the brainstem of chickens to trigger what he termed ‘complex behaviour sequences’ (von Holst, 1973; von Holst and von Saint Paul, 1960). For example, stimulation at one brainstem site reliably evoked ‘blinking of the left eye at first … occasional headshaking … then wiping the head against the shoulder, and finally scratching the left cheek with the foot’. Stimulation at another brainstem location instead triggered the following sequence: an end to feeding, tongue movements, salivation, neck stretching, shaking of the beak and then beak cleaning.

Jose Delgado also performed a variety of *in vivo* brain-stimulation experiments. Let us focus on a set of experiments in four monkeys he implanted with an electrode in the rostromedial part of the left red nucleus (Delgado, 1965). In each of these monkeys, when he stimulated for 5 s, he elicited a complex but stereotypical series of movements that far outlasted the stimulation, which he described thus: ‘…an immediate interruption of the animal’s spontaneous activities, change in facial expression, head turning to the right, standing up on two feet, circling to the right, walking on two feet with excellent preservation of equilibrium…, climbing the pole on the cage wall, and descending to the floor. Then, as after effects, the animal vocalized, adopted a threatening

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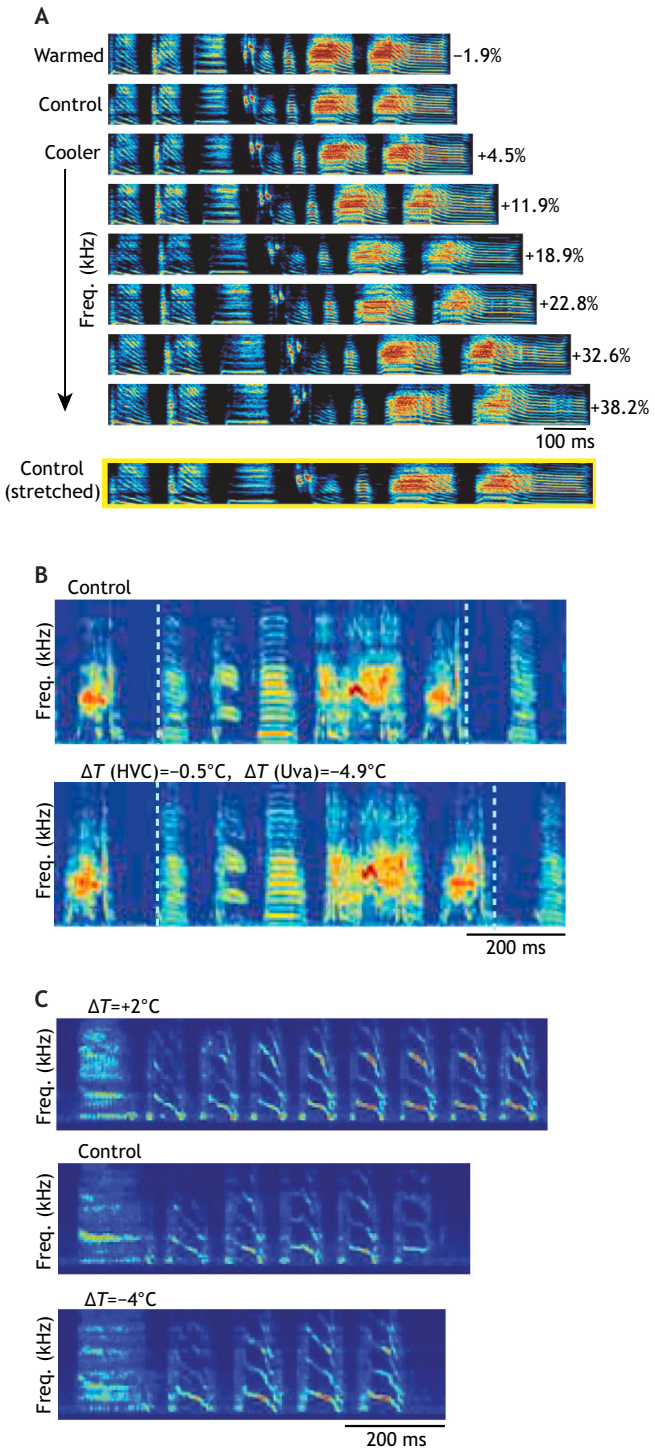

Fig. 5. Examples of songbird CNS temperature changes that alter song rate and sometimes syntax but otherwise preserve relative timing. (A) Warming (top sonogram) and progressive cooling (progressively lower sonograms) of HVC in a zebra finch speeds up and slows down, respectively, the rate of the song motif without altering the relative timing of syllables, as illustrated at the bottom by a temporally stretched control sonogram; percentages on the right indicate the change in motif duration. (B) Cooling the zebra finch Uva nucleus, even with little change in HVC temperature, also slows the song motif without changing the relative timing of syllables. (C) Warming (top) and cooling (bottom) HVC in Bengalese finches increases and decreases, respectively, the number of song syllables, i.e. it alters the syntax. Freq., frequency; *T*, temperature. A–C adapted from (A) Long and Fee (2008), (B) Hamaguchi et al. (2016) and (C) Zhang et al. (2017).

attitude directed toward subordinate monkeys…, walked a few steps on all fours, and peacefully approached some other member of the colony…'.

Delgado stated that he stimulated one of the four monkeys in this way over 20,000 times and elicited this entire movement sequence each time. This suggests that the electrical stimulation excited several brain networks that generate distinct, natural movements, as well as stereotypical coordination among them, with the movement sequence outlasting the stimulation.

Each of these findings of von Holst and Delgado is consistent with a set of individual-movement CPGs nested within a slower and overarching movement-sequence CPG. It may not be feasible to elicit (or interpret) motor patterns that mimic such a complex movement sequence in the absence of sensory feedback. That experiment would be the gold standard to demonstrate a CPG-

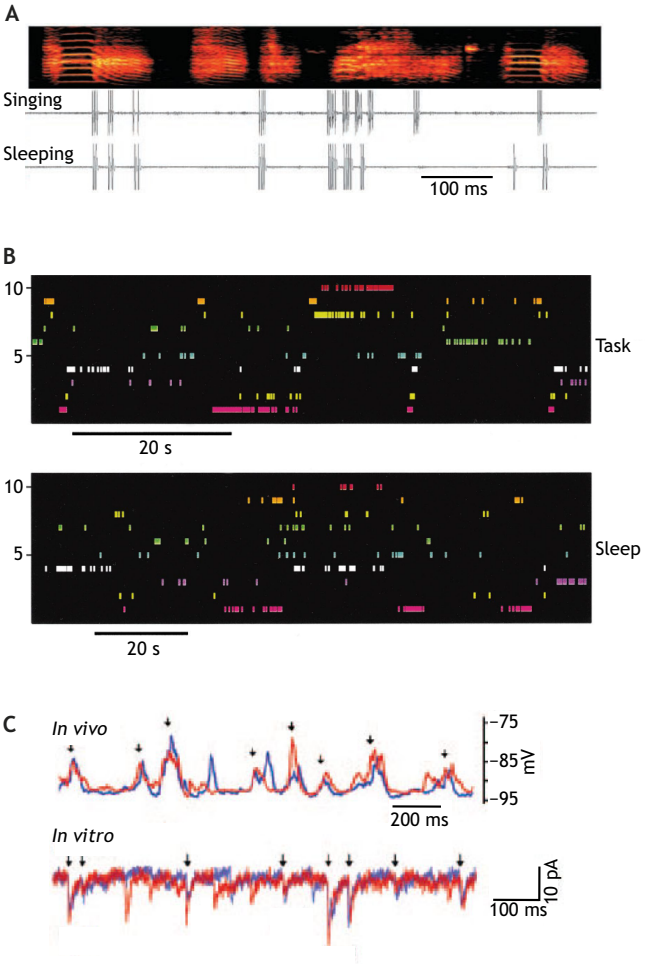

Fig. 6. Examples of stereotypical non-rhythmic activity sequences. (A) A sequence of spikes recorded extracellularly from a zebra finch RA neuron during singing (top, along with the sonogram) and later during sleeping (bottom). (B) Sequences of spikes from several (color-coded and numbered) rat hippocampal neurons recorded extracellularly while the rat traversed a track (Task, top) and later while it slept (Sleep, bottom). (C) Two superimposed sequences (red and blue) of postsynaptic potentials (PSPs) recorded intracellularly from cat visual cortex *in vivo* (top) and postsynaptic currents (PSCs) recorded intracellularly under voltage clamp from mouse visual cortex slices *in vitro* (bottom). Arrows indicate consistently timed PSPs and PSCs. A–C adapted from (A) Dave and Margoliash (2000), (B) Louie and Wilson (2001) and (C) Ikegaya et al. (2004).

driven movement sequence. If such an experiment is not feasible, perhaps one could still gather additional circumstantial evidence of the types I have highlighted here to suggest that such complex movement sequences are CPG driven.

Cortical CPGs for cognitive functions

In vertebrates, most applications of the CPG concept have been to the spinal cord and brainstem, where CPGs for simple, rhythmic movements are typically found. It has been argued, however, that the CPG concept can be usefully applied to the generation of rhythmic oscillations by large populations of mammalian cerebral cortical neurons (Traub et al., 2017; Yuste et al., 2005), including during primate reaching (Churchland et al., 2012). Cortical oscillations (see Glossary) may cause sensory inputs to have phase-dependent effects (Schroeder and Lakatos, 2009), i.e. different effects at different moments within the cycle of activity, as in the spinal cord (Burke, 1999; Duysens et al., 2013). There are also parallels between spinal and brainstem CPG-driven motor rhythms and both hippocampal and cerebellar population oscillations (Grillner et al., 2005). Also, the human cerebrum, in the absence of sensory cues and any instructed task, generates infra-slow CPG-like activity rhythms (Mitra and Raichle, 2016; Raichle, 2010), though perhaps via different mechanisms.

Cortical oscillations are in one sense just as simple as innate, rhythmic behaviors, such as locomotion and breathing, that were the initial focus of CPG research, as they involve sustained repetition of one cycle. In another sense, however, these oscillations are more complex, as they can be associated with higher cognitive functions, including perceptual binding of multiple sensory features of an object, working memory, attention and conscious awareness (Daitch et al., 2013; Rey et al., 2014; Thut et al., 2012; Uhlhaas et al., 2009; Wang, 2010), though others have suggested that such oscillations may not be functionally relevant and may be epiphenomena (Hermes et al., 2015; Roelfsema et al., 2004; Shadlen and Movshon, 1999; Thiele and Stoner, 2003). Another difference is that cortical oscillations are monitored not by motor output, but by summed activity of many cortical neurons, typically via local field potential (LFP) electrodes, electroencephalograms or functional magnetic resonance imaging. The divorce of these rhythms from motor output makes it more difficult to decipher their function. Regardless, CPG-like cortical circuits may generate these oscillations, which do not require but may be modulated by sensory inputs and chemical neuromodulators.

The most complex CPG-generated activity patterns may be stereotypical but non-rhythmic sequences of cortical electrical events. A major practical limitation is having a reliable and consistent output to monitor in the absence of a motor pattern or oscillatory cortical activity. But here, too, insights may be gained by turning to birdsong.

Sleeping birds precisely replay stereotypical firing sequences of RA neurons that occur during singing (Fig. 6A), demonstrating that these spontaneous activity sequences are both stereotypical and movement related, though no behavior or motor output occurs during sleep (Dave and Margoliash, 2000; Rauske et al., 2010). Similarly, hippocampal firing sequences in sleeping rats replicate sequences occurring during navigation, suggesting learned CPGs for navigation (Fig. 6B) (Louie and Wilson, 2001; Skaggs and McNaughton, 1996). Spontaneous cortical membrane potential sequences in mice *in vitro* and cats *in vivo* also repeat precisely (Fig. 6C) (Ikegaya et al., 2004, 2008), perhaps as a result of cell assemblies that generate chains of synchronized activity bursts (Buzsáki, 2010; Hebb, 1949; Huyck and Passmore, 2013). If one can rigorously relate monitored neuronal activity sequences to behavior or motor output in the same animal, one may define and study brain mechanisms of spontaneous complex stereotypical sequences, including whether they require sensory feedback, can be reset by electrical stimulation, or can be sped up or slowed down by warming or cooling particular structures. Such experiments may help narrow down locations and mechanisms of CPG-like complex but stereotypical activity sequences in the brain.

Conclusions and future directions

In this Review, I have tried to show that the CPG concept has been fruitfully applied to the study of complex but stereotypical activity sequences like birdsong, which contain distinct elements in a complex order. Such uses go beyond simple rhythmic motor patterns, like locomotion and respiration, that the CPG concept was originally created for. The CPG concept might also be applied to still more complex movement sequences like bird courtship displays and mammalian brain stimulation-evoked behavioral sequences. But is there value in such applications, or is this just a semantic statement with no practical consequence?

I suggest there may be value in thinking of stereotypical but complex activity sequences as potentially controlled by nested CPGs, or CPGs within CPGs (Fig. 4). This draws attention to the possibility of a CNS network generating each element, as well as an overarching CNS network that makes connections among networks to establish the relative timing of the elements, and mechanisms that trigger the overarching network to begin and end activity. Such thinking could influence the types of experiments performed, which could include attempts to reset an ongoing activity sequence, to warm or cool a particular CNS region, or to evoke the same activity sequence in a reduced preparation. Such approaches might narrow down the CNS region(s) generating the sequence, to be explored further using additional approaches. Such a CPG mindset may also draw attention to the extensive research on intrinsic and synaptic ion channel mechanisms that establish timing in traditional CPGs (Arshavsky et al., 2016; Harris-Warrick, 2010; Marder and Calabrese, 1996; Marder et al., 2014; Selverston, 2010) and the computational modeling of such CPGs that has revealed mechanisms likely to generate transitions between sequence elements (Ausborn et al., 2018; Grillner, 2006; Hao et al., 2011; Hull et al., 2016; Marder et al., 2014; Selverston, 2010; Shevtsova and Rybak, 2016). The roles of such mechanisms can then be explored for complex movement and non-motor activity sequences, which may increase our understanding of their neural control. If awareness of CPG research is increased among neurobiologists who would typically consider CPGs irrelevant to their research, this cross-fertilization may lead to creative hypotheses and experiments.

Acknowledgements

I thank the participants in the Patterning Neural Activity in the Central Nervous System satellite meeting in New Zealand for feedback on the initial presentation. I thank Douglas Gaffin, Michael Markham and four anonymous referees for comments on earlier versions of this manuscript.

Competing interests

The author declares no competing or financial interests.

Funding

National Science Foundation award IOS-1354522 provided financial support.

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