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A switch in aminergic modulation of locomotor CPG output during amphibian metamorphosis

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1. ABSTRACT

In the South African clawed frog, *Xenopus laevis*, a complete functional switch in the mode of locomotion occurs during development from axial, undulatory, tail-based swimming in post-hatching tadpoles to limb-based kick propulsion in the adult froglet. At key stages during the metamorphosis from tadpole to frog both locomotor systems are present, co-functional and subject to modulation by the two ubiquitous biogenic amines, serotonin (5-HT) and noradrenaline (NA), arising from the brainstem. Here we review evidence on the roles of 5-HT and NA in the early maturation and dynamic modulation of spinal locomotor circuitry in the postembryonic tadpole and describe the way in which the modulatory effects of the two amines, which are always in opposition, subsequently switch during the metamorphic period of development. We speculate on the underlying cellular, synaptic and network mechanisms that might be responsible for this change in role.

2. INTRODUCTION

In most animals, the rhythmogenic networks (so-called "central pattern generators" or CPGs) controlling locomotory behaviors, like walking, swimming or flying, appear early in development in order to equip the still immature organism with the ability to move efficiently through its environment, evade predation and survive into adulthood. The adaptability of these locomotor movements is an essential requirement which relies upon an intrinsic flexibility in the motor output generated by CPGs within the central nervous system. In turn, this flexibility at the level of the rhythmic activation of motor neurons, the final common pathway underlying all movements, is largely imparted by a plethora of modulatory control systems that sculpt a basic CPG-driven locomotor rhythm into one that is appropriate to environmental contingencies and developmental requirements. An important constraint that modulatory systems must confront is the fact that the body size and format generally change dramatically as an animal

matures. In creatures that undergo development within the context of a single body format, where essentially a small adult emerges from the egg or the mother, the changes involved are primarily qualitative and involve a mere scaling of the body as maturation progresses. Such is the case with many fish, birds, a few amphibians and also mammals and whose nervous systems must accommodate the gradually changing biomechanical requirements of the locomotor system. However, for the majority of anuran amphibians, such as the South African clawed frog *Xenopus laevis*, an altogether different set of challenges must be faced when a qualitative scaling of body size is also associated with a quantitative switch in body format during the metamorphic phase of their development.

Metamorphosis of the anuran tadpole to frog constitutes one of the most dramatic developmental transformations in biology, involving massive alterations in body structure, a transfer from aquatic to aerial respiration, a switch from herbivorous to carnivorous diet, and biochemical, physiological and morphological changes in virtually all of the animal's organs (1). With respect to locomotion, the change in body plan from tadpole to adult is accompanied by the growth of limbs and the regression of the tail as the organism alters its locomotory strategy from the employment of undulatory, tail-based swimming in larvae to limb-based kick propulsion in the young adult. These biomechanical modifications to the locomotory system during metamorphosis require a dynamic anatomical and functional restructuring of underlying neural circuitry within the central nervous system. Such a remodeling must include the appearance of new sensory and motor pathways to service the emerging limbs, in direct contrast to the loss of precursor larval spinal circuitry that accompanies the regression and eventual loss of the tail. Therefore, the gradual replacement of the tail-based swim network by adult limb-kick circuitry during the metamorphic process implies that addition, deletion and functional reassignment of spinal neuronal elements are occurring simultaneously. Moreover, during this transitional developmental period, in which a primary locomotory system is being replaced by a completely different one, the controlling influences of supraspinal modulatory systems must also be in the process of changing as they switch their downstream allegiance from axial to the de novo limb spinal circuitry before the former disappears with tail resorption.

As for a variety of vertebrates, including chicks (2), cats (3) and rodents (4-6), the locomotor CPG networks of *Xenopus* are subject to the modulatory actions of a variety of neurotransmitters, including the monoamines, 5-HT and NA, conveyed mainly by descending projections from the brainstem. Although these two neuromodulatory systems have been well characterized, are phylogenetically conserved and have anatomically defined origins, primarily but not exclusively in the raphe nuclei (5-HT) and *locus coeruleus* (NA), the way in which they modulate the components of locomotor systems is multifaceted and bewilderingly complex. Each system makes extensive, diffuse contacts with both motor and sensory circuitry in the spinal cord where their effects on distinct neuronal and synaptic targets are mediated via the activation of multiple metabotropic receptor sub-types that in turn couple to divergent intracellular second messengers and their downstream effectors. Moreover, these same modulatory inputs must deal with the perpetually changing nature of the neural circuits on which they impinge in order to satisfy the changing demands of locomotor behavior during ontogeny.

In this review we survey the evidence that: i) 5-HT and NA exert potent, but opposing, modulatory actions on locomotor swimming activity in post-hatching *Xenopus* tadpoles; ii) 5-HT is additionally involved in the post-embryonic maturation of the swimming CPG; and iii) during the subsequent metamorphic transition in locomotor strategy from larval tail-based swimming to adult hindlimb kick propulsion, there is a complete functional switch in the roles of the two amines in tadpoles versus froglets. Our understanding of each of these topics is still superficial, but enough information has been gained to enable speculation on how aminergic modulation orchestrates the adaptive operation of amphibian locomotor CPG circuitry.

3. *XENOPUS* LOCOMOTION: MATURATION, METAMORPHOSIS AND MODULATION

3.1. Ontogeny of *Xenopus* swimming behavior

As in many animals, locomotor networks are assembled and become functional extremely early in *Xenopus* development, even before the embryo emerges from the egg membranes. In fact, the first rhythmic and rudimentary propulsive movements can be observed in embryos released from the egg from around stage 28 (7), a mere 32 hours post-fertilization and long before normal eclosion which occurs at approximately 50 hpf (stage 37/38). At this time the newly hatched tadpole is generally dormant but when touched on the trunk or tail it will swim efficiently and for up to many seconds using body bends that alternate between the two sides and propagate tail-wards at 10 to 20 Hz (8, see 9 for recent review). The tadpole remains mainly sessile for a few days until around stage 43-45 at which time it joins the water column and swims more or less continuously for the remainder of its filter feeding larval existence. The hindlimb buds first appear in pre-metamorphic larvae at stage 49, and from the onset of pro-metamorphosis at stage 53 until stage 58, they become increasingly motile, although locomotion is still exclusively axial with the hindlimbs, that are still insufficiently developed to participate in active propulsion, being held extended against the body (10). By metamorphic climax, at stage 60-61, the hindlimbs have increased both in size and motility and they now participate actively in body propulsion via bilaterally synchronous cycles of limb extensions (power-stroke phase) that alternate with bipedal flexions (return-stroke phase). Until the tail is resorbed (by stage 64), when locomotion becomes exclusively appendicular, the transitional animal is still able to employ axial swimming (at cycle frequencies of 5 to 15 Hz), either alone or in combination with the much slower (0.5 to 3 Hz) rhythmic kicking movements (10,11; see below).

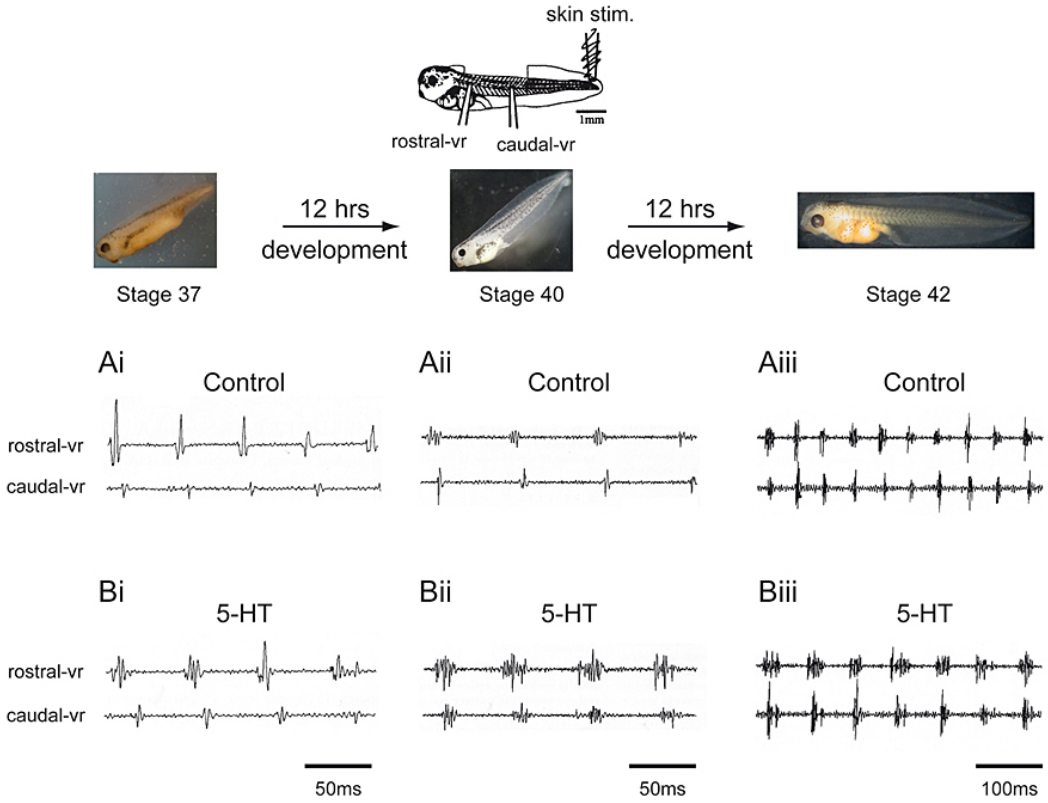

Figure 1. Rostrocaudal development of spinal locomotor discharge in post-embryonic *Xenopus* tadpoles (A) and effects of bath-applied 5-HT (B). Ai, In immobilized hatchling embryos (stage 37/38), recordings of rostral and caudal ventral roots (vr) show a single spike occurring on each cycle of fictive swimming evoked by a brief electrical stimulus of the tail skin (see schematic at top). Aii, 12 hours later (at stage 40) motor impulse bursts now occur rostrally while single spikes still occur caudally. Aiii, 1 day after hatching (stage 42), all segments are able to produce a burst of action potentials per swim cycle, indicating a progressive rostrocaudal maturation of the axial CPG network. Bi, 5-HT applied to a stage 37/38 embryo causes bursting in rostral, but not caudal roots (cf. control stage 40 in Aii). Bii, 5-HT enhances rostral bursts and converts single caudal spikes into impulse bursts (cf. control stage 42 in Aiii). Biii, at stage 42, 5-HT enhances ongoing bursts in both rostral and caudal segments. Reproduced with permission from (18).

3.2. Early development of larval CPG output and the role of 5-HT

The neural control of swimming in stage 37/38 hatchling tadpoles (Figure 1A) has been studied intensively over the past 30 years and is now well understood. In particular, with the advent of patch clamp recordings and neurobiotin labeling of spinal cord and brainstem neurons, the anatomical and functional organization of the swimming CPG has been elucidated in unprecedented detail, but is beyond the scope of the present review (see 9,12 for recent synopses). During episodes of fictive swimming recorded in immobilized animals, the CPG's output consists of a relatively stereotyped motor pattern suitable to drive actual swimming movements (Figure 1Ai), consisting of brief, ca. 5 msec duration ventral root activity that alternates between the left and right sides and propagates rostrocaudally at 10 to 20 Hz. The motor discharge per cycle is brief due to near simultaneous firing of a single action potential in each of the dozen or so myotomal motor neurons that innervate each hemi-segment, which are coupled by electrical synapses promoting synchronicity (13).

Dramatic changes in the locomotor output occur during the first day of larval life; although the basic coordination of the fictive swimming at stage 42 (ca. 24 hours post-hatching) remains unchanged, the duration and variability of ventral root activity increases (Figure 1Aiii) (14). This change is due to changes in the firing properties of spinal motor neurons (15,16) which can now discharge action potentials multiply on each cycle and, probably, an accompanying decrease in the strength of electrical coupling between members of individual motor pools to enable de-synchronized firing (13). The maturational acquisition of a more variable CPG output, which in turn imparts greater efficiency and flexibility to actual swimming behavior, follows a rostrocaudal progression with longer bursts initially occurring rostrally but briefer, embryo-like bursts still being expressed more caudally (Figure 1Aii) (14). This suggests the influence of a modulatory signaling pathway that itself progressively descends the spinal cord during early larval development.

In many vertebrate systems, including mammals, the descending projections of serotonergic raphespinal

interneurons reach the spinal cord around the time of birth or hatching and this is also the case for *Xenopus* tadpoles (17). Initial circumstantial evidence for a causal link with the change in the swimming rhythm structure derived from the finding that bath-applied 5-HT mimics in a stage-specific manner the normal rostrocaudal appearance of locomotor bursts during early larval development (18). Thus, at hatchling stage 37/38 when raphe serotonergic projections have only reached the rostral spinal cord, exogenous 5-HT enhances rostral but not caudal ventral root discharge (Figure 1Bi). At stages 40 and 42, however, when serotonergic pathways have extended progressively more caudally, 5-HT increases burst durations along the length of the animal (Figure 1Bii, Biii). A causal link between the ingrowth of serotonergic spinal innervation and the maturation of larval swimming was supported by the additional finding that ablation of raphespinal projections in pre-hatching embryos with a serotonin neurotoxin prevented subsequent locomotor development, leading to larvae that remained capable of generating an embryo-like swimming rhythm only (19). These findings are consistent with a large body of evidence that 5-HT can play a developmental, trophic role in the assembly and maturation (and indeed the repair) of neural circuits, in addition to the more acute changes that this amine triggers via effects on the properties of neurons and synapses in the spinal cord.

3.3. Dynamic modulation of larval swimming by 5-HT and NA

Once the more flexible "bursty" rhythm has developed at larval stage 42 it continues to be susceptible to short-term serotonergic modulation (18,20). 5-HT receptor activation increases the intensity and duration of axial motor bursts and reduces swim episode durations, but has a less obvious effect on the frequency of the swimming rhythm. These influences of 5-HT have been partially explained by activation of 5-HT_{1A} receptors (21), but it is likely that 5-HT activates other sub-types simultaneously. An increase in motor burst duration is a common feature of 5-HT modulation in vertebrate motor networks but in several other model systems an accompanying reduction in locomotor frequency is observed (e.g. lamprey and zebrafish; 22,23). One possible explanation is that the receptor subtypes and downstream targets vary between the different systems. For example, a major target of 5-HT in the lamprey is the slow post-spike afterhyperpolarization (sAHP): 5-HT reduces the sAHP amplitude and the resulting spike accommodation, enabling neurons to fire at higher frequency and for longer in each cycle (22) . A sAHP is not evident in recordings from hatchling *Xenopus* tadpole neurons and although the Ca^{++}-dependent K^{+} current that normally underlies it is absent at this stage in *Xenopus* neurons, it appears during early larval development such that it is detectable in stage 42 neurons (24). This gradual onset of the current early in larval development may explain the relative lack of effect of 5-HT on rhythm frequency compared with other vertebrates, but subsequently it is likely to play a more prominent role in the serotonergic modulation of locomotion, as indeed is suggested by the amine's pronounced lengthening influence on cycle period of *in vitro* fictive swimming in later stage pre-metamorphic tadpoles (see below). Similarly, the slowing effect of 5-HT on lamprey swimming has been partly explained by inhibition of glutamate release via activation of a 5-HT_{1D} receptor (25) which is present in early larval *Xenopus* neurons (26) but may not be fully expressed.

In contrast to 5-HT, noradrenaline (NA) has a prominent slowing effect on the swimming rhythm when bath-applied (27) but with no clear effect on the absolute duration of motor bursts in each cycle. These NA effects are mediated by activation of alpha-adrenoreceptors (28). At the level of the network output then, 5-HT and NA appear to exert overall opposing influences on rhythm generation with 5-HT leading to a relatively short, intense bouts of locomotor output whereas NA produces a slower, weaker swimming motor pattern. What are the underlying neuronal targets of the two amines in the central nervous system, and to what extent do their actions overlap?

3.4. Cellular and synaptic sites for 5-HT and NA modulation

Although the list of aminergic targets in the *Xenopus* locomotor system is far from complete, the modulation of inhibitory synapses appears to be importantly involved (27). Specifically, 5-HT and NA exert differential and opposing control over the amplitude of the mid-cycle glycinergic IPSPs mediated by commissural interneurons that are rhythmically active during swimming and which couple the left and right sides of the cord in antiphase (Figure 2A). Thus, mid-cycle IPSPs during swimming are facilitated by NA (Figure 2Bi) and reduced or blocked by 5-HT application (Figure 2Bii). Moreover, these opposing effects of the two amines appear to be mediated presynaptically to alter the glycine release mechanism itself, since 5-HT and NA respectively decrease (Figure 2Ci) and increase (Figure 2Cii) the rate of occurrence of spontaneous tetrodotoxin-resistant IPSPs, without affecting their amplitudes. Can these effects explain the opposing global influence of the amines on swim frequency? Although the evidence is correlative rather than causative, previous pharmacological and computer simulation studies suggest that the strength of mid-cycle inhibition is an important determinant of swim frequency: weakening transmission (e.g. through exposure to strychnine, or by simulating reduced inhibitory conductances) increases swimming frequency, while strengthening inhibition (e.g. via allosteric modulation of glycine receptors) (KT Sillar, unpublished observations), or turning up the inhibitory conductance, has the opposite effect of slowing the swimming rhythm. A working hypothesis then is that 5-HT increases burst durations, at least in part, by reducing mid-cycle inhibition to allow neurons to fire earlier and for longer in each cycle, while NA increases cycle periods by strengthening the mid-cycle inhibition and delaying the onset of the next cycle. A direct test of this hypothesis is still needed and it should also be remembered that other unidentified targets of the amines could well be important in rhythm modulation. The effects on glycinergic transmission, whilst very clear and measureable, may simply be a parallel consequence rather than the actual or sole source

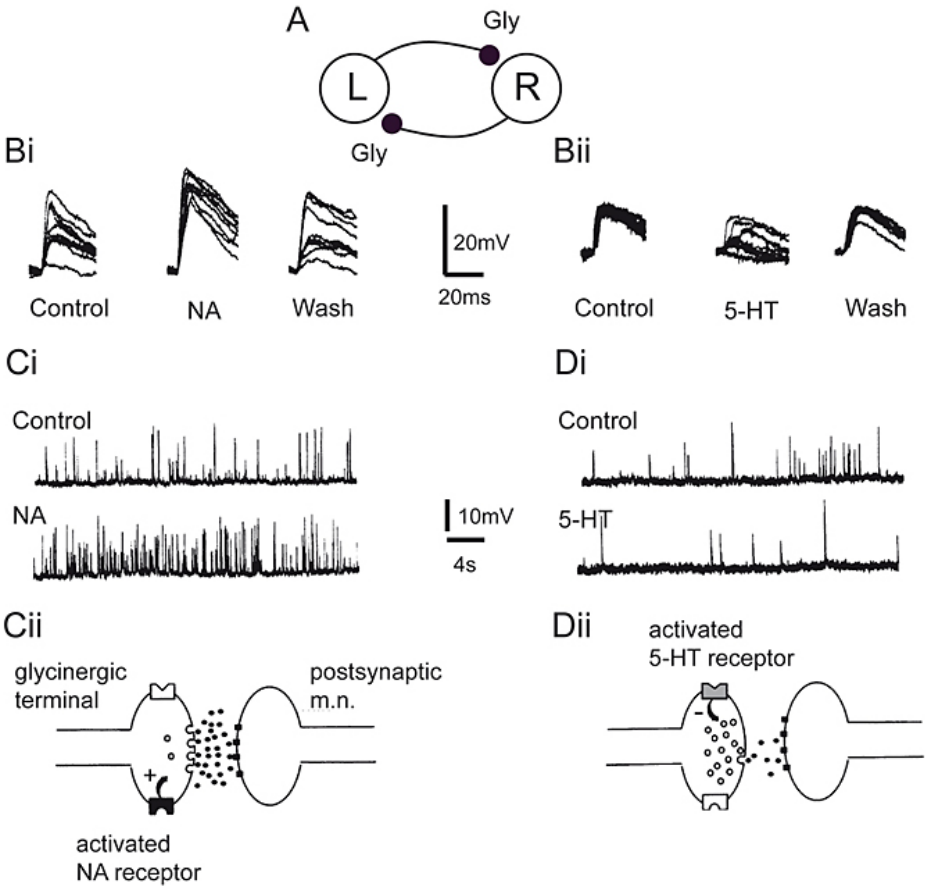

Figure 2. 5-HT and NA exert opposing modulator actions on glycinergic synaptic inhibition by commissural interneurons. A,. Schematic of the reciprocal glycinergic inhibitory connections that couple the left (L) and right (R) sides of the spinal cord in anti-phase during swimming. B, NA enhances (Bi), while 5-HT reduce (Bii) the amplitude of midcycle IPSPs during swimming. (Note, IPSPs are depolarizing due to injection of chloride into neurons from the recording microelectrode). Several consecutive cycles of swimming are superimposed in control, amine and wash conditions. Ci, Di, NA and 5-HT increase and decrease, respectively, the frequency of TTX-resistant spontaneous glycinergic IPSPs but do not affects their amplitudes. Cii, Dii,. Schematics showing proposed pre-synaptic locations of NA and 5-HT receptors on terminals of commissural interneurons, which differentially couple to the glycine release machinery. Reproduced with permission from (27).

of the two amines' modulatory actions on swimming motor output.

3.5. Opposing 5-HT and NA modulatory actions switch during metamorphosis

The effects of 5-HT and NA in early stages in the life of *Xenopus* tadpoles clearly differ from those described in other adult vertebrates like the lamprey (22), zebrafish (23) and cat (3). There are two non-mutually exclusive explanations for this difference: i) phylogeny – there is a species-specific reason why the amines differentially affect locomotion in *Xenopus* versus other vertebrates; and ii) ontogeny – the effects suit the niche of younger *Xenopus laevis* tadpoles, but switch during development to match the effects seen in other adult vertebrates. Up until stage 42 the *Xenopus* tadpole is an essentially sessile organism that spends its time dormant, often attached via its rostral cement gland to an object in the environment. Presumably this is an anti-predatory strategy to remain less visible, out of sight of predators that are tuned to the detection of moving targets, while other bodily systems are maturing. However, shortly afterwards at around stage 43-45, once the mouth has opened and the gut has developed, the tadpole rights itself and becomes free swimming and filter feeding in the water column. The animal is now larger and the tail more powerful, so it is presumably better equipped and more manoeuvrable to avoid predation.

The way in which these two amines modify locomotion during later stages of *Xenopus* development has been examined in more recent studies on the acquisition of the limbs and the loss of the larval axial locomotor system during metamorphosis (10,29). During this critical transitional period, the freely behaving animal is capable of different modes of locomotion according to how far and how fast it wishes to swim, with both the co-existing, primary tail-based and secondary limb-based systems participating either independently or conjointly in

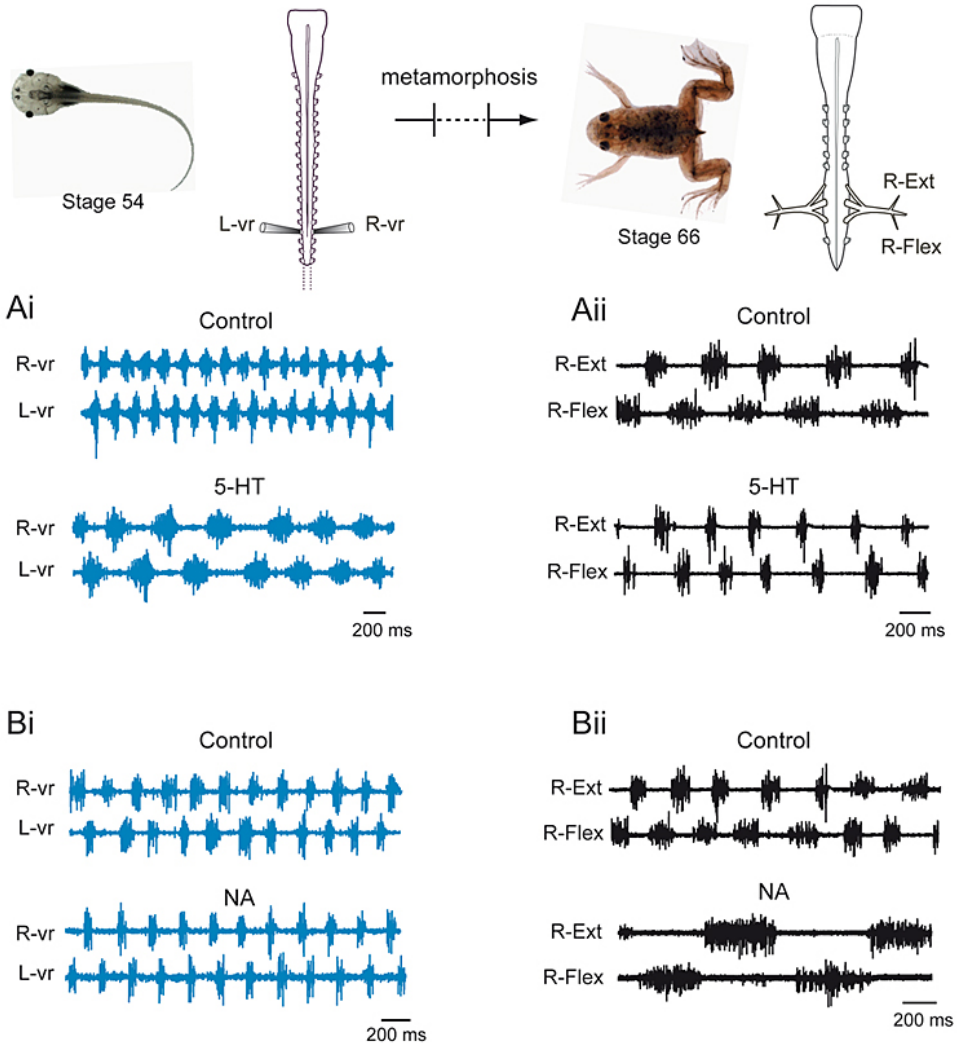

Figure 3. Differential and opposing modulation of locomotor CPG networks by 5-HT and NA before and after metamorphosis. Ai, 5-HT applied to the isolated brainstem/spinal cord of pre-metamorphic larvae (upper schematic), modulates spontaneous axial CPG activity by enhancing the durations and decreasing the cycle periods of spinal ventral root bursts. Aii, In contrast, 5-HT applied to *in vitro* preparations (upper schematic) from post-metamorphic froglets when the tail has regressed and limb-based swimming is exclusively used, decreases burst durations and cycles periods of spontaneous appendicular CPG activity. Bi, Bii, Broadly opposite effects of exogenous NA. In the pre-metamorphic tadpole (Bi), NA decreases axial burst durations but without significant effects on cycle periods. In the post-metamorphic froglet (Bii), NA now enhances burst durations and cycle periods of appendicular network activity. Reproduced with permission from (29).

swimming behavior. In the latter condition, moreover, the two locomotor modes may be expressed in distinct rhythms with very different frequencies, or when higher velocities are required, tail oscillations and rhythmic hindlimb kicking occur at the same frequency in a cooperative propulsive action (10,11). Importantly, the motor output patterns that drive such variably coordinated behavioral rhythms continue to be spontaneously expressed by the underlying pattern-generating networks in the completely isolated CNS (10), thereby offering the opportunity to explore the coordination between two neighboring CPG circuits, the extent and functional consequences of which can be readily recognized both *in vivo* and *in vitro*.

At late larval stages (50-54) when the tadpole is free swimming, but before the emergence of functional limbs, the spontaneous fictive swimming rhythm recorded in isolated spinal cord-brainstem preparations is reminiscent of the stage 42 rhythm in its basic coordination (bilateral alternation and a brief rostrocaudal intersegmental delay), but the increase in burst duration that accompanied postembryonic development has continued with bursts in each cycle lasting up to ~5 times longer (Figure 3Ai, upper panel). At these pre-metamorphic stages, application of 5-HT now has an effect resembling that which is found in adult axial-based swimmers, like the lamprey (22) and the zebrafish (23), producing a large increase in burst durations in each cycle and accompanied by a very prominent

reduction in locomotor frequency (Figure 3Ai, lower panel) (29). In contrast, NA applied at these stages has little or no effect on swimming frequency but, significantly, it reduces burst durations (Figure 3Bi). Therefore the two amines continue to exert opposing modulatory actions, but the effects have completely switched with respect to swim frequency.

The ensuing stages of development witness the dramatic and complete switch in locomotor strategy from an exclusively axial-based system to an appendicular system with hindlimb extension-flexion cycles providing the propulsive thrust for swimming. As described above, in the intervening stages the limbs become progressively more functional, but with the tail still present such that the hybrid animal at metamorphic climax can use both systems coincidently, or interchangeably. We have identified two epochs with respect to the gradual emergence of the secondary hind limb circuitry from the pre-existing spinal axial CPG (10). At stage 57 the limbs are small and have acquired motility, but contribute little to locomotor thrust compared to the larger, more dominant tail. Ventral root recordings from *in vitro* preparations revealed that the extensor and flexor nerves of the limbs are rhythmically active, but in time with the axial rhythm and, in contrast to their later coordination, express synchronous bursts within one side and in cross-cord alternation. Thus the initial limb network is functionally coupled to the axial system and follows the axial rhythm as a slave oscillator. From stage 59 until the metamorphic climax, however, the limb network extricates itself functionally (and perhaps also anatomically) from the axial system to participate actively in body propulsion. Now the two rhythms adopt completely different cadences (the limb rhythm is far slower than the axial rhythm) and they can occur independently or coupled together (see Figure 4Ai and 4Aii, respectively). In the latter case the conjoint rhythm is expressed at a frequency somewhere in between the two independent rhythm frequencies. Moreover, in contrast to the earlier combined rhythm expressed at pro-metamorphosis, the appendicular patterns are themselves coordinated appropriately for generating independent hindlimb kick cycles, with ipsilateral flexor and extensor motoneuron bursts occurring in phase-opposition, and with homologous bilateral motoneurons bursting synchronously.

Now the effects of the two amines become even more complex and intriguing. In preparations that are spontaneously generating independent axial- and limb-based rhythms at their different intrinsic frequencies (Figure 4Ai), 5-HT slows down the axial system (as it did at pre-limb stages), but accelerates appendicular rhythmicity (Figure 4Aii). In so doing, 5-HT not only exerts an opposite influence on the two networks, it also induces a cycle-by-cycle coupling of the otherwise separate patterns into a single harmonized rhythmic output that is identical in the two networks. In contrast, preparations that are already displaying the conjoint rhythm (Figure 4Bi) respond oppositely to noradrenergic modulation: by differentially increasing and decreasing the cycle frequencies of axial and limb motor burst activity, respectively, NA's presence now leads to a decoupling into two separate rhythms (Figure 4Bii), precisely the opposite to the effects of 5-HT. Once the metamorphic climax has taken place and the tail has degenerated, the propulsive machinery is exclusively limb-based. In froglets 5-HT and NA have the same differential effects that were displayed in the hybrid, mid-metamorphic organism on the appendicular system: 5-HT accelerates the locomotor rhythm with a corresponding reduction in burst durations thus maintaining a constant duty cycle (Figure 3Aii); NA on the other hand slows down the limb-kick rhythm and increases the cycle period, once again maintaining the proportion of each cycle in which the locomotor bursts occur (Figure 3Bii).

The finding that 5-HT and NA modify the developmentally independent rhythms expressed before (Figure 3Ai, Bi) and after (Figure 3Aii, Bii) metamorphosis in a similar manner to the amines' coupling/decoupling effects on the adjacent fast axial and slow appendicular circuits during metamorphosis (Figure 4) therefore indicate that the differential aminergic actions are targeted both at the level of inter-circuit connectivity, as well as the cellular and synaptic properties within the individual networks themselves (29). Interestingly, opposing monoaminergic modulation of neuronal circuitry has been described in other motor systems, including the enhancement (by 5-HT) or depression (by NA) of mammalian respiratory activity (30) and the opposing facilitation of recurrent inhibition (by 5-HT) or excitation (by NA) in spinal motor pathways (31). Although the amines' similar "push/pull" control of the adjacent locomotor networks in metamorphosing *Xenopus* can be seen to satisfy the immediate and changing propulsive requirements of the animal, it remains to be determined whether 5-HT, as at early post-embryonic stages (see above), and/or NA are additionally involved in the maturational shaping of spinal circuit operation during this critical secondary phase of development.

3.6. Implications of nitrergic brainstem signaling

A further important source of supraspinal modulatory influence on larval *Xenopus* locomotor circuitry during development is nitric oxide (NO), a gaseous signaling molecule linked to diverse ontogenetic processes, including neurogenesis, cell differentiation, proliferation and apoptosis (32-35). Our earlier findings are also consistent with a multi-faceted physiological and developmental role of NO in the modifications to *Xenopus* spinal circuitry during metamorphosis (36). In pre-metamorphic tadpoles, NO-synthase (NOS) expression is restricted to neurons in the brainstem (37,38), but NOS containing neurons appear in the spinal cord from the onset of the metamorphic period (36). Interestingly, NOS staining is at first lacking from regions of the spinal cord where the *de novo* limb motor neurons are located suggesting that NO may have an early negative influence on network development, so that its absence from the cervical and lumbar regions of the early metamorphosing cord enables the neuronal proliferation required for the initial assembly of limb circuitry (36,39). At subsequent metamorphic stages, when NOS expression becomes more evenly distributed throughout the spinal cord (36), it is possible that NO switches to a permissive regulatory role,

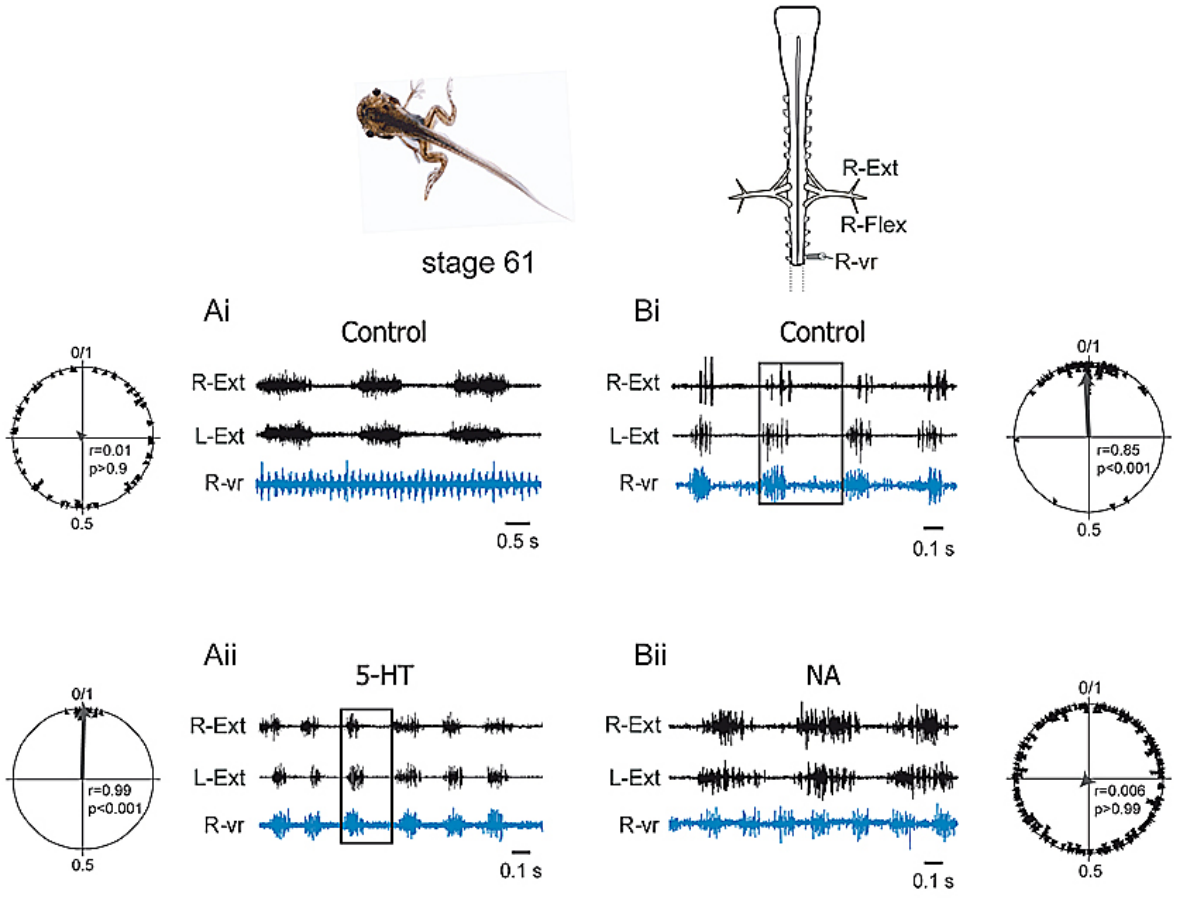

Figure 4. Opposing influences on rhythm parameters and functional coupling of axial and appendicular circuitry by 5-HT and NA during metamorphosis. At metamorphic climax (stage 61) when the axial and limb CPG networks coexist in the same animal, 5-HT applied to the isolated brainstem/spinal cord (upper schematic) combines otherwise independent network rhythms (Ai) into a single coordinated rhythm (Aii). This coupling is indicated by the strongly significant phase preference of R-vr versus R-Ext burst onsets in the corresponding polar plot at left of Aii, cf. circular phase plot at left of Ai, left. In contrast, NA applied to isolated preparations already spontaneously expressing coordinated network activity (Bi, see phase plot of R-vr versus R-Ext burst onsets at right) decouples the two networks to allow independent rhythm generation (Bii). Reproduced with permission from (29).

enabling the late-phase refinement of circuit synaptic wiring, as found elsewhere in the developing nervous system (e.g. 40).

In addition to this potential longer-term role during ontogeny, NO also acutely modulates locomotor output in newly hatched *Xenopus* larvae, decreasing the swim frequency and swim episode durations (37,38) and thus having a net inhibitory effect on swimming. These actions are mediated in part by NO's facilitation of fast inhibitory transmission. Specifically, NO is thought to directly potentiate descending GABAergic inhibition from the brainstem (to shorten swim episodes) and to enhance glycinergic inhibition in the spinal cord via an indirect activation on the brainstem NA system (41). Preliminary data suggest that NO continues to affect locomotor activity during metamorphosis, but once again in the opposite direction to earlier larval stages, activating both axial and limb motor rhythms at pro-metamorphic stages (D Combes, K Sillar, J Simmers; unpublished observations).

In summary, NO functions as an inhibitory "metamodulator" of locomotor output at the mainly sessile early larval stages, before the onset of free swimming, by shifting the balance of influence of the 5-HT and NA modulatory systems with opposing actions on swimming towards the noradrenergic form of motor output (41). During metamorphosis the two amines also exert opposing modulatory actions on the expression of tail and limb rhythms, albeit influences that become inverted compared to earlier, pre-metamorphic stages of development. Perhaps NO differentially regulates the interactions between these two spinal locomotory systems via the dynamic metamodulation of descending aminergic pathways, as well as orchestrating the developmental switch in the latter's opposing influences during the metamorphic process.

4. CONCLUSIONS AND PERSPECTIVE

The actions of 5-HT and NA on the locomotor networks of *Xenopus* tadpoles change dynamically and in

parallel with the dramatic changes in the organization and expression of rhythmic motor output sub-serving axial and limb-based movements. Moreover, we have found that the effects of the two amines are opposing at any given stage but that their roles switch between early post-hatching animals and later free-swimming stages.

Although the basic effects of the two amines at the level of the motor output have now been described, we have only just begun to scratch the surface with regard to their actions at the cellular and synaptic levels. This represents an important area for future study because so many important questions remain unanswered. These include: i) when and where in the networks are the amine receptors that mediate the observed affects expressed and how does this expression alter during ontogeny?; ii) which second messenger systems are the receptors coupled to and how does the modulation of their downstream targets affecting firing properties and synaptic transmission account for the overt influences on locomotor activity?; iii) how can we explain the developmental switch in effects in terms of receptor activation and ion channel modulation within and between the axial and limb networks?; and iv) what is the hierarchical organization of the aminergic modulatory systems and NO metamodulation in the short and long term effects of the amines during development?

Understanding fully the computational logic and operational plasticity of neural networks necessarily derives from detailed knowledge of cellular and molecular mechanisms, as well as the organizational rules that govern a given circuit's assembly and remodeling during the course of ontogeny. To this end, a combination of genetic approaches with electrophysiological investigation in mice has already pinpointed some of the molecular developmental processes that regulate the differentiation and organization of identified populations of spinal neurons with specified locomotor network functions (42-44). Moreover, since similar molecular and organizational principles also appear to be engaged in the ontogeny of spinal swim circuitry in zebrafish (45), it is plausible that an ancestral, phylogenetically-conserved template for the assembly of embryonic spinal motor systems exists as a general developmental feature of vertebrates (46,47). In the case of *Xenopus*, however, the assembly of limb circuitry occurs at a relatively much later stage in development, with this secondary network emerging from within the framework of an already mature and fully functional axial circuit, and with the two systems having to co-exist and operate within the same organism before the former gains functional independence and the latter disappears. The extent to which molecular signaling cascades responsible for the delayed configuration of limb circuitry differ from, and interact with, those underlying primary axial network construction remains to be determined, as does the developmental substrate for the parallel realignment and actions of their upstream modulatory pathways as one locomotor system gradually supplants the other.

Thus, by lying at the interface between two of the principal locomotor strategies in the animal kingdom, *Xenopus laevis* not only constitutes an excellent experimental model for studying how central networks are assembled and organized during ontogeny (10,29,48,49), but also offers a unique opportunity to gain insights into the evolutionary rules by which the localized CPG networks for vertebrate limb-based locomotion have emerged from the segmentally-distributed circuitry of their undulatory swimming ancestors (43,44). Moreover, future cellular studies comparing the supraspinal control of CPG circuitry in the tadpole with that of the frog, and particularly during the transitional metamorphic period, should enable deciphering common phylogenetic principles and differences in the mechanisms by which convergent modulatory systems, such as those utilizing biogenic amines, orchestrate both the short-term adaptive flexibility and long-term development of spinal networks. In this respect, the recent establishment of the full genome sequence of closely related *X. tropicalis* (50) will eventually offer the exciting opportunity to generate specific, genetically modified strains that are amenable to addressing a vast array of questions through the acute manipulation of molecularly-defined populations of neurons with optogenetic approaches (51).

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Abbbreviations: CPG: central pattern generator, 5-HT: serotonin, NA: noradrenaline, sAHP: slow post-spike afterhyperpolarization, IPSP: inhibitory postsynaptic potential, NO: nitric oxide, NOS: nitric oxide synthase

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