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REVIEW

Influence of long-term social interaction on chirping behavior, steroid levels and neurogenesis in weakly electric fish

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Summary

Social interactions dramatically affect the brain and behavior of animals. Studies in birds and mammals indicate that socially induced changes in adult neurogenesis participate in the regulation of social behavior, but little is known about this relationship in fish. Here, we review studies in electric fish (*Apteronotus leptorhychus*) that link social stimulation, changes in electrocommunication behavior and adult neurogenesis in brain regions associated with electrocommunication. Compared with isolated fish, fish living in pairs have greater production of chirps, an electrocommunication signal, during dyadic interactions and in response to standardized artificial social stimuli. Social interaction also promotes neurogenesis in the periventricular zone, which contributes born cells to the prepacemaker nucleus, the brain region that regulates chirping. Both long-term chirp rate and periventricular cell addition depend on the signal dynamics (amplitude and waveform variation), modulations (chirps) and novelty of the stimuli from the partner fish. Socially elevated cortisol levels and cortisol binding to glucocorticoid receptors mediate, at least in part, the effect of social interaction on chirping behavior and brain cell addition. In a closely related electric fish (*Brachyhypopomus gauderio*), social interaction enhances cell proliferation specifically in brain regions for electrocommunication and only during the breeding season, when social signaling is most elaborate. Together, these studies demonstrate a consistent correlation between brain cell addition and environmentally regulated chirping behavior across many social and steroidal treatments and suggest a causal relationship.

Key words: neurogenesis, communication, social behavior, cortisol, electric fish.

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Introduction

The contemporary study of adult neurogenesis has it origins in work on communication behavior. In the 1980s, Fernando Nottebohm and colleagues showed that changes in the songs of canaries correlated with addition of neurons in a brain region (the high vocal center) that controls vocal behavior (Goldman and Nottebohm, 1983; Alvarez-Buylla et al., 1988). Since then, many studies in vertebrates have shown that social interaction influences cell proliferation and neurogenesis in the brain (Lieberwirth and Wang, 2012; Gheusi et al., 2009; Font et al., 2012; Almli and Wilczynski, 2009; Barnea and Pravosudov, 2011). However, given the complexity of social signaling in most species, it is often difficult to identify specific features of social interaction that are effective for influencing neurogenesis. Moreover, the brain regions that show adult neurogenesis are usually embedded in complex networks and are only indirectly connected to the production of communication signals. Thus it is difficult to describe the precise role of new neurons in contributing to behavioral change.

In electric fish, however, these problems are simplified. Electric fish are unusually good models for investigating the link between the social environment and neurogenesis because brain regions controlling communication signals have single functions and their activity is closely connected to the behavioral output of the whole animal. For example, neurons in the prepacemaker nucleus (PPn), a region that controls certain electrocommunication signals, are only two synapses removed from the final effector cells that generate the communication signal. Because the PPn has only one predominant output, the behavioral relevance of new neurons added to this nucleus during adulthood can be determined with relative ease. Another advantage of electric fish is that components of social interaction can be separated readily because their primary mode of social signaling, electrocommunication signals, is relatively simple and thus easily manipulated and presented experimentally. Thus, electric fish are quite useful for dissecting out the specific components of social interaction responsible for enhanced adult neurogenesis.

Here, we begin by briefly describing an electrocommunication behavior termed chirping and its neural control. Then we review our work showing that long-term social interaction (1–2 weeks) between electric fish (*Apteronotus leptorhynchus*) increases both the production of chirps and the addition of new cells to the brain, particularly near the region that regulates chirping. By manipulating the social stimuli presented to the fish, we demonstrate that dynamic and novel social stimuli are most effective in enhancing both the rate of chirp production and cell addition. We describe experiments showing that cortisol mediates, at least in part, the effect of long-term social interaction on chirping behavior and brain cell addition. Finally, we summarize our studies of a closely related electric fish (*Brachyhypopomus gauderio*) demonstrating that social interaction enhances cell proliferation specifically in electrocommunication regions of the brain only during the breeding season, when social activity is highest. Our

field studies further show that, beyond these regionally and seasonally specific effects of social interaction, additional complexities in natural environments elevate cell proliferation non-specifically across the whole brain. The consistent correlation between changes in chirping behavior and cell addition across experiments using several kinds of social stimuli suggests a causal relationship between behavioral change and neurogenesis in electric fish.

Introduction to chirping behavior and its neural control

Many species of gynmnotiform electric fish emit a continuous, wave-type electric organ discharge (EOD) that they use for electrolocation and electrocommunication. Generally, the baseline EOD frequency is remarkably stable (Moortgat et al., 1998). However, fish can modulate their EOD to produce social signals that vary in frequency change and duration (Zakon et al., 2002). Chirps are brief frequency elevations that are emitted in both aggressive and courtship encounters. In *A. leptorhynchus*, chirps come in two main forms: Type 1 chirps have large frequency excursions (~500 Hz), are long in duration (~25 ms) and predominate in opposite-sex interactions; and Type 2 chirps have relatively small frequency excursions (~100 Hz), are short in duration (~15 ms) and predominate in male–male interactions (Bastian et al., 2001; Zakon et al., 2002; Engler et al., 2000).

In isolation, fish chirp spontaneously at a low rate, with chirp rate higher at night than during the day (Zupanc et al., 2001; Dunlap et al., 2011a). When male fish are paired with another male, they increase chirp rate dramatically within seconds, producing almost entirely Type 2 chirps. In such dyadic interactions, fish often display an 'echo response' in which fish alternate chirps with ~200 ms delay between chirps (Hupé and Lewis, 2008; Hupé et al., 2008; Salgado and Zupanc, 2011).

Chirps can also be elicited by presenting fish with artificial sine wave stimuli that, in some ways, mimic the EOD of a conspecific fish. In one common experimental setup, fish are placed in a testing apparatus, a 'chirp chamber', and presented with stimuli whose frequency and amplitude can be easily controlled and standardized (Zupanc and Maler, 1993; Larimer and MacDonald, 1968). In both direct dyadic interactions and chirp chamber responses, chirping is highly sexually dimorphic, with males producing chirps at 10–20 times higher rates than females (Dunlap, 2002; Zupanc and Maler, 1993; Dulka et al., 1995).

A primary determinant of chirp rate is the frequency difference (dF) between the fish's own EOD and the EOD of the stimulus fish (or the artificial sine wave stimulus): small dFs elicit higher chirp rate than large dFs. Chirp rate is also influenced strongly by stimulus amplitude (Bastian et al., 2001; Dunlap et al., 1998), and, because EOD amplitude attenuates drastically through the water, the distance between fish determines chirp production (Dunlap and Larkins-Ford, 2003; Hupé and Lewis, 2008; Zupanc et al., 2006): higher stimulus amplitudes and shorter distances between fish elicit higher chirp rates. In both dyadic interactions and chirp chambers, chirp rate habituates relatively quickly after the onset of stimulus, with chirp rates declining to half-maximal values after ~10 min of stimulation (Dunlap, 2002; Harvey-Girard et al., 2010). In summary, chirp rate is influenced by the sex of the focal fish, the frequency difference and proximity of the stimulus fish, the duration of the interaction and the time of day.

Chirping is generated by a paired, diencephalic nucleus, the prepacemaker nucleus (PPn), which is a lateral extension of the central posterior thalamic nucleus (CP) located ~400 μm lateral to the ventricle. The CP/PPn receives inputs from several sensory and telencephalic brain regions and projects strongly through monosynaptic output to the hindbrain pacemaker, which controls the EOD frequency (Zupanc, 2002). Activation of the PPn accelerates firing of the pacemaker nucleus through glutamatergic synapses, causing to the fish to emit chirps (Kawasaki et al., 1988; Dye, 1988).

Chirp rate is influenced by a large range of neurochemicals and hormones (Zupanc, 2002). For example, exogenous treatment of fish with arginine vasotocin (Bastian et al., 2001), noradrenaline (Maler and Ellis, 1987), androgens (Dunlap et al., 1998; Dulka et al., 1995) or cortisol (Dunlap et al., 2006) increases chirp rates, while treatment with serotonin inhibits chirping *via* the 5HT2 receptor (Maler and Ellis, 1987; Telgkamp et al., 2007; Smith and Combs, 2008).

Long-term social interaction and chirping behavior

Most studies of chirping behavior have examined chirping response to social stimuli on relatively short time scales from 1 to 15 min. Here, we review our research on chirping on a longer time scale, examining the effect of paired interactions for 1–2 weeks on chirp production and the chirp response to standardized sinusoidal stimuli. All these experiments used paired males (*A. leptorhynchus*) of similar size (within 1–2 g) and EOD frequency (20–30 Hz) separated by mesh barriers that prevented fish from injuring each other through aggression but permitted communication through electric, olfactory and visual modalities. Although we did not examine chirp structure systematically, virtually all (>95%) chirps in such interactions were Type 2 chirps.

Chirping during dyadic interactions

When two males are introduced to the same tank, chirping varies along two time scales (Fig. 1). Initially, there is an immediate burst of chirping that lasts 36 h, during which time chirp rate in paired fish is approximately five to 20 times greater than in isolated fish (Dunlap et al., 2011a). For the next 5.5 days, chirp rate fluctuates on a diurnal cycle, with paired fish chirping at approximately three times the rate of isolated fish during both the day and night. Such social interaction does not affect EOD frequency or locomotion, demonstrating that social interactions do not simply generically stimulate electromotor or motor output.

Maximal chirp rates in these dyadic interactions are lower than those commonly reported for fish exposed to sine wave stimuli in a chirp chamber. This is likely due to the fact that: (1) in pairs, fish can swim freely, which often reduces the effective amplitude of the stimulus to below that typically presented to fish in a chirp chamber (Dunlap and Larkins-Ford, 2003), and (2) the difference frequency in our experiments is usually 20–30 Hz, while in chirp chamber studies, the dF is typically less. Fish in dyadic interactions appear to habituate their chirp rate more slowly than fish in chirp chambers: in pairs, fish chirp at elevated rates for 36 h (Dunlap et al., 2011a) while those presented periodically with sine wave stimuli every 2 min habituate completely after only ~1.5 h (Harvey-Girard et al., 2010).

What specific stimuli influence chirp production during long-term interactions? In real social interactions, fish experience EOD stimuli that vary in amplitude and waveform as they swim around in the tank and engage in reciprocal chirping. Several experiments indicate that these stimulus dynamics and the exposure to another fish's chirps are important in determining both the initial chirp rate and the long-term rate of habituation. First, fish presented with a constant sine wave stimulus that does not contain chirps or amplitude and waveform variability chirped at rates that were

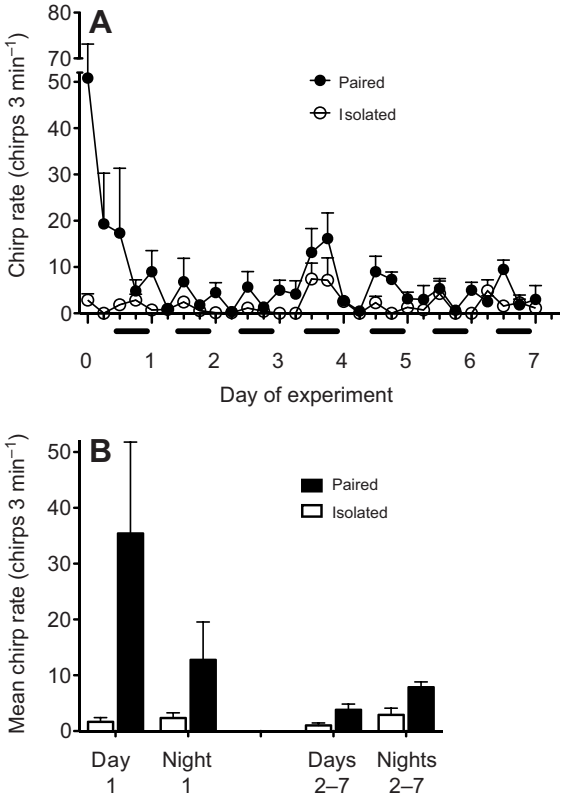

Fig. 1. (A) Time course of chirping in isolated (open circles) and paired male *Apteronotus leptorhynchus* (filled circles) over 1 week. Bars under the *y*-axis indicate the period of lights out. (B) Two phases of chirp production in isolated (white bar) and paired (black bar) fish. Paired fish chirp significantly more than isolated fish in the day and night of both phases. Redrawn from Dunlap et al. (Dunlap et al., 2011a).

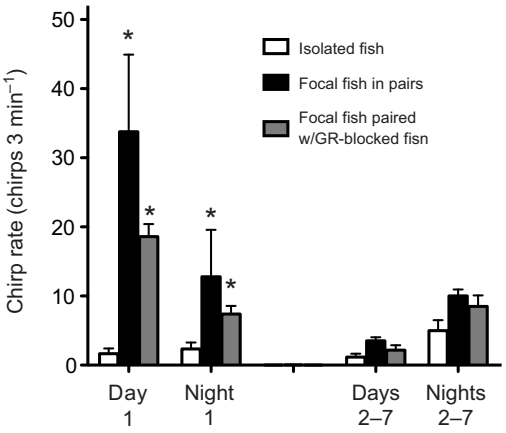

Fig. 2. Pharmacological inhibition of chirping in stimulus fish (*Apteronotus leptorhynchus*) decreases chirping in focal fish. The graph shows chirp rates in isolated fish (white bars), fish paired with a stimulus fish that was implanted with a blank capsule (black bars) and fish paired with a stimulus fish that was implanted with a glucocorticoid receptor (GR) blocker, RU486 (grey bars). Capsules were implanted in a manner identical to that described previously (Dunlap et al., 2011a). This administration of RU486 reduces chirp rate to levels found in isolated fish. Asterisks indicate significant differences from all other treatment groups (P<0.05).

intermediate in level between those of paired and isolated fish during the initial 36 h; for the remainder of the week, they showed full habituation (chirp rates equivalent to isolated fish) (T. Haught and H. Loring, unpublished data). Thus, full expression of socially induced chirping in both phases appears to depend on dynamic EOD stimuli and/or its active modulations.

A second experiment further suggested that the chirp rates of a focal fish in the first 36 h are influenced by chirp rate of the stimulus fish (Fig. 2). We treated stimulus fish with a compound (RU486, see details below) that lowered chirp rate to background levels without affecting other behaviors (i.e. EOD frequency and locomotion). Untreated focal fish paired with stimulus fish whose chirp rates had been reduced pharmacologically chirped at lower rates initially than fish paired with a sham-treated male with undisturbed chirp rates, suggesting that the chirp rates of focal fish depend on the chirp rates of the stimulus fish. We cannot rule out the possibility that this reduction in chirp rate was due to some other pharmacologically altered stimulus feature (e.g. olfactory signal) in the stimulus fish. However, in short-term interactions, chirping of the focal fish is quantitatively correlated (Dunlap, 2002) and temporally structured in an 'echo' pattern (Zupanc et al., 2006; Hupé and Lewis, 2008) with chirping in a stimulus fish. Thus it is likely that pharmacological manipulation of the stimulus fish decreased long-term chirp rates in focal fish by decreasing chirp stimuli from the stimulus fish. Interestingly, in the 5 days following this initial encounter, fish paired with an RU486-treated partner chirped at rates equivalent to those of fish exposed to a sham-treated partner, indicating that the degree of chirp habituation is unrelated to the partner's chirp rate.

A third experiment showed that the reduction in chirp rate in the second phase of interaction can be partially reversed by presentation with a novel social partner (Dunlap and Chung, 2012). Fish were paired with two or seven partners introduced sequentially and at regular intervals over 14 days. At each presentation of a new stimulus fish, the magnitude of the difference frequency was kept constant, but the sign (+dF *versus* –dF) was reversed such that the focal fish was aware of the presence of a novel stimulus fish (Harvey-Girard et al., 2010). Overall, fish exposed to two partners chirped more than fish paired with a single partner, and fish exposed to seven partners chirped more than the other two groups. These differences in overall chirp rate were due to bursts of elevated chirping following the introduction of each novel partner, demonstrating that a new stimulus fish can dishabituate chirping in the focal fish. Thus the long-term chirp rate appears to be determined strongly by degree of novelty in the social environment and not simply by the presence of another fish.

Chirping to artificial social stimuli

While the studies above indicate that presentation of social novelty can partially reverse the degree of chirp habituation, studies using standardized artificial signals indicate that long-term social interaction can potentiate chirping. Males housed in pairs chirped at greater rates than isolated fish when the stimulus fish was temporarily removed and the focal fish was tested in a chirp chamber (Fig. 3A). This effect emerged only after 6–7 days after pairing and was subsequently lost after 10 days of pairing. This indicates that, within the 6–10 day window, social interaction enhances the sensitivity of the neural circuitry underlying chirp production. This is the same time course as that of socially enhanced cell addition into the PPn (Fig. 3B) (see below), suggesting that socially induced changes in chirping behavior and neurogenesis may be causally related.

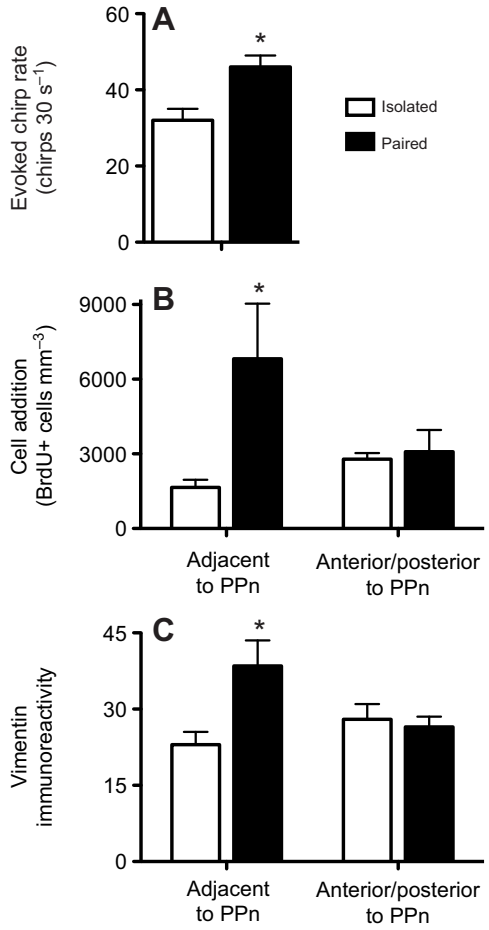

Fig. 3. Chirping behavior and regionally specific addition of cells and radial glial fibers in *Apteronotus leptorhynchus* that were isolated (white bars) and paired (black bars) for 1 week. (A) Chirp rate in response to sine wave stimuli presented in a chirp chamber after 1 week. (B) Cell addition in the periventricular zone (PVZ) adjacent to the central posterior thalamic nucleus/prepacemaker nucleus (CP/PPn) and in surrounding PVZ regions. (C) Density of radial glial fibers, as measured by vimentin immunoreactivity, in the PVZ. For information on vimentin quantification, see Dunlap et al. (Dunlap et al., 2006). Asterisks indicate significant differences from the isolated treatment group (P<0.05). Redrawn from Dunlap et al. (Dunlap et al., 2002; Dunlap et al., 2006).

Steroidal regulation of chirping behavior

As with many vertebrate communication behaviors, electrocommunication in weakly electric fish is influenced by steroid hormones. In *A. leptorhynchus*, androgen treatment enhances chirp rate in gonadectomized males (Dunlap et al., 1998) and intact females (Dulka et al., 1995) presented artificial stimuli. Similarly, plasma androgen [11-ketotestosterone (11-KT)] concentrations correlate positively with chirp rate during short-term (15 min) dyadic encounters between males (Dunlap, 2002), However, long-term social interaction between males, which potentiates chirping, has no effect on plasma 11-KT levels (Dunlap et al., 2002). Furthermore, chirp (Type 2) rate in males living long term in a mixed-sex social setting with environmental conditions mimicking the breeding season is unrelated to 11-KT levels in males with high EOD frequency and even negatively related to 11-KT levels in males with low EOD frequency (Cuddy et al., 2012). Thus, it appears that long-term social modulation of chirp rate, at least of Type 2 chirps, is not under androgen regulation.

Instead, cortisol appears to play an important role in mediating the effects of long-term social interaction on chirping behavior (Dunlap et al., 2002; Dunlap et al., 2011a). Plasma cortisol levels rise in males paired with males for a week, the same time course over which pairing potentiates chirping behavior. Implanting exogenous cortisol in isolated fish causes similar potentiation of chirping behavior (Fig. 6A). Blocking glucocorticoid receptors in paired fish using the antagonist RU486 reduces chirp rates to levels found in untreated isolated fish (Fig. 6D), indicating that glucocorticoid receptor binding is crucial for socially induced chirp behavior (Dunlap et al., 2011a). Furthermore, glucocorticoid receptor blockade inhibited not only the initial chirping response, but also the subsequent circadian pattern of chirping.

Long-term social interaction and brain cell addition

In the 1990s, Zupanc and colleagues mapped areas of cell proliferation throughout the adult brain of *Apteronotus* (Zupanc and Horschke, 1995) and in the diencephalon of *Eigenmannia* (Zupanc and Zupanc, 1992). They demonstrated that cells are born at especially high rates in the periventricular zone (PVZ), and some of these cells subsequently migrate to the CP/PPn and differentiate into neurons. With this background, we examined whether long-term social interactions, which were known to potentiate chirping, also enhanced cell proliferation and neuronal differentiation in axial regions of the PVZ that contribute cells to the CP/PPn. In all the studies discussed below, we injected BrdU in fish 3 days before they were killed and quantified BrdU+ cell density. In our initial study (Dunlap et al., 2006), we examined both males and females and found no difference in their proliferative response to social interaction; thereafter we used only males. We use the term ‘cell addition’ to refer to the two-part process of cell proliferation and 3 day survival. This survival period was chosen because previous work showed that 3 days is adequate for some cells born in the PVZ to begin lateral migration and neuronal differentiation (Zupanc and Zupanc, 1992).

Pairing fish for 1 week enhanced PVZ cell addition in a regionally and temporally specific manner. Pairing increased cell addition in the PVZ adjacent to the CP/PPn, but not in the surrounding control regions (Fig. 3B) (Dunlap et al., 2006). This regional specificity indicates that social interaction does not influence the brain globally *via* an overall effect on metabolic rate or growth rate. Rather, cell addition in the region that donates cells to the CP/PPn is particularly sensitive to social stimuli. Moreover, social enhancement of cell addition is temporally specific, coinciding with the period that chirping behavior is potentiated. That is, paired fish have higher rates of cell addition and evoked chirping than isolated fish at 7 days after treatment and do not differ from isolated fish in either measure at 1, 4 or 14 days after treatment (Dunlap et al., 2006; Dunlap and Chung, 2012). Paired interaction does not affect swimming behavior (Dunlap et al., 2011a), and thus the effects of social interaction on cell addition are not secondary to socially enhanced exercise, as has been demonstrated in mammals (van Praag et al., 1999; van Praag, 2008). The regional and temporal specificity is consistent with the hypothesis that cell addition could be a mechanism or consequence of changes in chirping behavior, but as discussed below, a direct causal relationship has not yet been established.

In a follow-up set of experiments, we sought to determine the fate of cells born in the PVZ during social stimulation. Quickly after pairing (within 1 day) radial glia begin increasing the extent of their fibers that span laterally from the PVZ (Fig. 3C) (Dunlap et al., 2006), providing a potential migratory pathway for newborn cells (Zupanc and Clint, 2003). Within a week, approximately 60% of

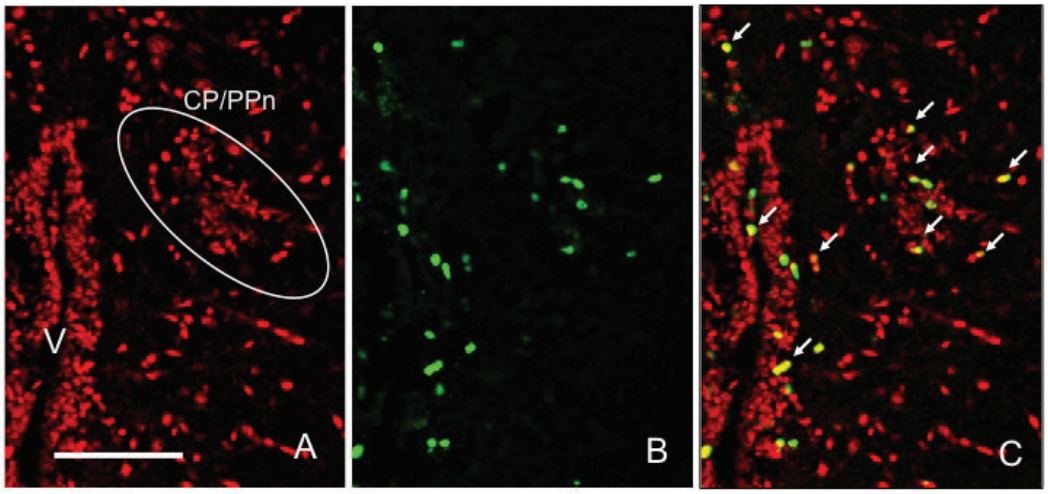

Fig. 4. Neurogenesis in the CP/PPn of *Apteronotus leptorhynchus*. (A) Section labeled with fluorescent Nissl stain (NeuroTrace) showing all neurons. (B) Section labeled with anti-BrdU showing all cells added over a 3 day period. (C) Merge of A and B, showing double-labeled newborn neurons marked with arrows, some of which are present in the CP/PPn. Scale bar (applies to all panels), 100 µm. Modified from Dunlap et al. (Dunlap et al., 2008). V designates the ventricle of the brain.

the cells born in the PVZ differentiate into neurons, and BrdU+ cells with clear neuronal phenotype are found in the CP/PPn over the time course in which pairing affects chirping (Dunlap et al., 2008) (Fig. 4B). Importantly, many cells born in this PVZ region do not become neurons in the CP/PPn, and one interesting avenue of research is to examine the full range of fates (including apoptosis) of cells born under social stimulation.

The relative simplicity of electrocommunication as a mode of social interaction enabled us to identify specific social stimuli that are sufficient for promoting cell addition (Dunlap et al., 2008). Fish were housed in separate aquaria connected by wires that allowed one-way delivery of the EOD of one fish to the aquarium of another fish. Fish receiving such stimuli had equivalent levels of PVZ cell addition as those living in pairs, indicating that unimodal (electric), non-reciprocal signaling is sufficient to fully induce social enhancement of cell addition (Fig. 5A). However, constant sine wave stimuli of the same mean amplitude and difference frequency were completely ineffective: fish presented with these constant stimuli had rates of cell addition equivalent to those of isolated fish (Fig. 5B). The effective EOD stimuli differed from ineffective sine wave stimuli in only three ways: waveform (quasi-sinusoidal EOD *versus* pure sine wave), amplitude dynamics (variable *versus* constant) and the presence of chirps (rare, spontaneous chirps *versus* no chirps). Through synthetic playbacks, we can now begin to identify which of these three features of real electrocommunication signals are necessary and sufficient stimuli to promote adult neurogenesis.

The positive effects of social interaction described above result from 1 week of social pairing. However, as mentioned previously, fish paired for a longer period (2 weeks) show rates of cell addition similar to those of isolated fish, indicating that they habituate to constant social stimuli. Such habituation can be prevented by sequential introduction of novel partner fish (Dunlap and Chung, 2012). When fish are presented with a novel partner after 1 week, their rates of cell addition after 2 weeks are similar to those living with a partner for 1 week. Moreover, fish presented sequentially with seven novel social partners over 2 weeks add more cells than fish presented with two partners for 2 weeks or a single partner over any duration. Thus, just as with long-term chirp rate, the rate of cell addition is positively related to the amount of social change and not simply the presence or absence of social stimuli.

Steroidal regulation of brain cell addition

The studies summarized above demonstrate that long-term social interaction simultaneously affects chirping behavior and brain cell addition near the CP/PPn, but what mechanisms underlie these socially induced changes? Several studies indicate that cortisol contributes to promoting socially induced cell addition while also regulating chirping behavior. One week of exogenous cortisol treatment elevates cell addition specifically in the PVZ adjacent to the CP/PPn over the same time course that it potentiates chirping (Fig. 6A,B). Moreover, blocking glucocorticoid receptors pharmacologically with RU486 causes an opposite effect: simultaneous inhibition of cell addition and socially induced chirping (Fig. 6D,E).

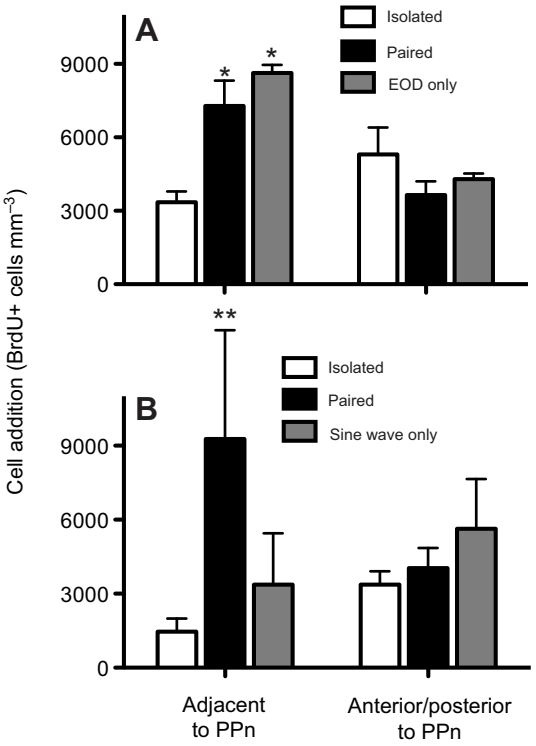

Fig. 5. (A) Cell addition in *Apteronotus leptorhynchus* is stimulated in a regionally specific manner and to the same degree in paired fish and fish only receiving the EOD of another fish in an adjacent tank. (B) Constant sine wave stimuli are completely ineffective in promoting cell addition. *Significantly different from isolated treatment group; **significantly different from both isolated and sine wave only treatment groups (P<0.05). Redrawn from Dunlap et al. (Dunlap et al., 2008).

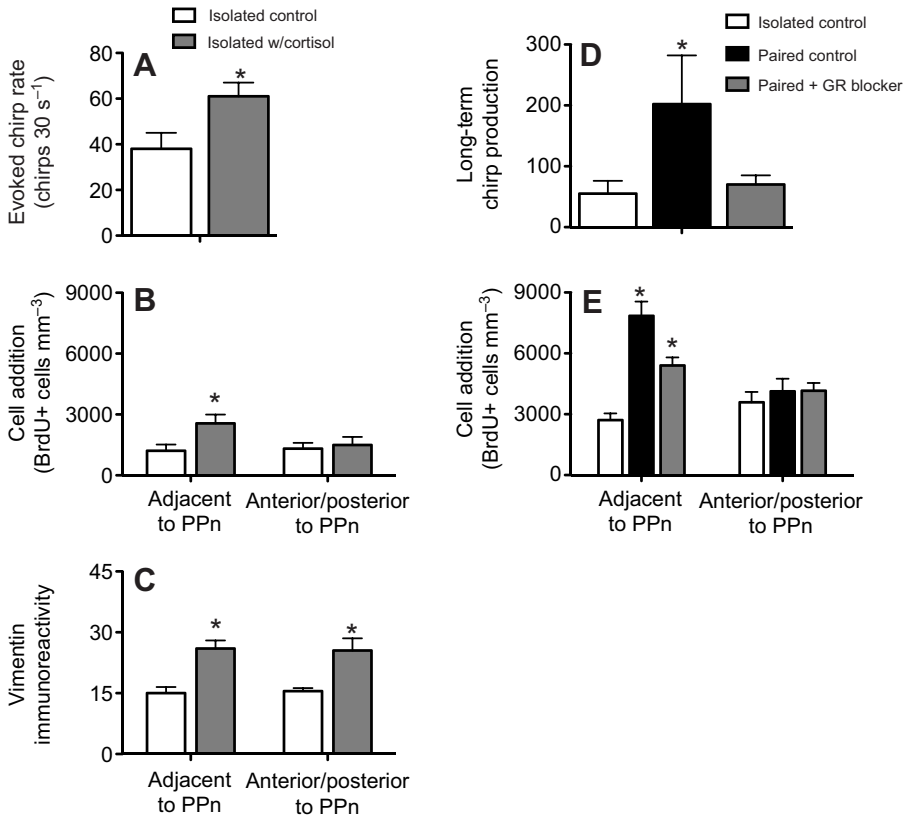

Fig. 6. Effect of 1 week treatment with cortisol (A–C) and the glucocorticoid receptor (GR) blocker RU486 (D,E) on chirping behavior and periventricular cell addition and radial glia in *Apteronotus leptorhynchus*. (A) In isolated fish, chirp rate in response to sine wave stimuli is potentiated by implantation of cortisol compared with implantation with a blank capsule (control). (B) Regionally specific cell addition in the PVZ near the PPn is stimulated by implantation with cortisol. (C) Radial glial formation is stimulated in a regionally non-specific manner by cortisol implantation. (D) Total chirp production exhibited during 29 sampling periods (3 min each) distributed equally during the day and night over 7 days. Glucorticoid receptor blockade reduced chirp production to levels displayed by isolated controls. (E) Glucocorticoid receptor blockade partially inhibits cell addition in the PVZ near the PPn in a regionally specific manner. Asterisks indicate significant differences from other treatment groups (P<0.05). Redrawn from Dunlap et al. (Dunlap et al., 2011a; Dunlap et al., 2006; Dunlap et al., 2002).

However, certain differences between the response of hormone-manipulated fish and untreated fish indicate that the effect of cortisol is less specific and less potent than social interaction on plasticity of the PVZ. First, cortisol treatment causes non-specific increases in radial glia formation across the PVZ after 1 week and cell addition after 2 weeks. Second, cortisol treatment is only about half as effective as social interaction in promoting cell addition (Fig. 6B,E). Also, unlike the effect of GR blockade on chirping, where the effect is complete (Fig. 6D), the effect on cell addition is only partial. Cell addition in RU486-treated, paired fish is still elevated above that of isolated fish (Fig. 6E). These observations suggest the following model: socially induced elevation of cortisol exerts a broad influence across the PVZ, including the area adjacent to the CP/PPn, but other activity dependent factors (e.g. brain-derived neurotrophic factor) act in combination with cortisol to augment cell addition near the CP/PPn.

Social and environmental influences on brain cell proliferation in a natural population of electric fish

Our studies on *A. leptorhynchus* demonstrate a strong effect of social interaction on brain cell addition. However, the relevance of these studies to natural phenomena might be limited because the social manipulations we made in the laboratory are very simple (isolated *versus* paired), the spatial dimension of the social interactions is very small (38 l aquaria) and the reproductive state of the fish is usually quiescent.

To address how fish are influenced by more natural stimuli and physiological cycles, we examined brain cell proliferation in the closely related gymnotiform electric fish, *Brachyhypopomus gauderio*, living in natural and semi-natural environments and experiencing seasonal reproductive cycles (Dunlap et al., 2011b). We compared male fish living isolated in the laboratory, fish living in groups of 12–16 fish in large outdoor enclosures and fish living in their natural habitat, a lake in northern Uruguay. Fish were injected with BrdU 2 h before they were killed, giving us a measure of cell proliferation rate. Compared with isolated fish, fish living in complex social groups showed greater rates of cell proliferation in the PVZ adjacent to the CP/PPn, but not in the PVZ of other mid-brain regions or other hindbrain proliferative zones. This corroborates our work in *Apteronotus* showing a regionally specific influence of social interaction on cell dynamics in the brain. However, we found that this social enhancement of proliferative rates occurred only in the breeding season, when testes are mature and social electrical signaling, including chirping, is most elaborate. Moreover, other brain regions devoted exclusively to electrogenesis (the pacemaker) and electrosensation (the electrosensory lateral line lobe) showed similar seasonally specific enhancement of cell proliferation. The observation that cell proliferation is elevated by group living only in brain regions associated with electrocommunication and only during the season when electrocommunication is most elaborate suggests that new brain cells might contribute to seasonal changes in communication behavior or that increased social electric signaling may enhance proliferation of new cells in these specific brain regions.

Beyond these regionally specific effects, the natural environment and breeding season had large global effects on cell proliferation throughout the midbrain and hindbrain. Across all seasons and brain regions examined (including the electrocommunication brain regions mentioned above), fish living in the wild produced new brain cells at rates approximately two to five times higher than captive fish. We could not identify the precise feature of the natural environment that causes this dramatic difference, but we hypothesized that the spatial and social complexity of the natural habitat plays a role. In addition, fish during the breeding season, regardless of their social

environment and reproductive state, produce cells at rates three to seven times higher than those during the non-breeding season, and we hypothesized that this effect is largely attributable to seasonal changes in temperature or day length.

Summarizing the correlation between cell addition and chirping

Thus far, our research has shown consistent correlated changes in chirping behavior and cell addition near the CP/PPn across many different social and hormonal manipulations. To summarize, after fish (*Apteronotus*) are paired for 1 week, cell addition increases specifically near the CP/PPn, coinciding with the time at which chirping is potentiated. After 2 weeks of pairing, both cell addition and chirp rate decrease to baseline levels found in isolated fish, but introduction of novel fish during these 2 weeks simultaneously increases both cell addition and chirp rate in a 'dose-dependent' manner. Presentation of artificial social stimuli, which are less effective in stimulating chirping, fail to promote cell addition. Cortisol treatment simultaneously enhances cell addition and potentiates chirping, while blocking glucorticoid receptors inhibits both cell addition and long-term chirp production. Finally, in *Brachyhypopomus*, cell addition near the CP/PPn is elevated in seasons, reproductive states and social settings when chirps rates are highest.

These correlations suggest but do not prove a causal relationship between cell addition and chirping behavior. Social and hormonal manipulation could change both brain and behavior independently. Moreover, even if cell addition and chirping are causally related, the direction of causality is not clear. That is, social enhancement of cell addition could be the cause or the effect of changes in chirping. Establishing such causal relationships will require developing techniques to experimentally ablate neurogenesis in the CP/PPn and chirp production in socially interacting fish.

Questions for further research

Although it is clear that adult-born cells differentiate and survive as neurons in the CP/PPn, the activity and precise fate of these newborn neurons is not understood. Because the CP/PPn is viable in a slice preparation, it is becoming feasible to record from newborn neurons while stimulating with chirp-inducing stimuli (Zakon, 2006). To understand the contribution of newborn neurons to chirp production, it will also be crucial to characterize their synapses and connectivity within the CP/PPn and determine whether they project to the pacemaker nucleus.

Our work has focused on the possible role of cells born near the CP/PPn in regulating chirping behavior. However, it is important to note that cells born in this PVZ region doubtlessly migrate to other regions of the brain (e.g. hypothalamus) and could influence other behaviors or neural processes. Conversely, chirping is influenced by other brain regions whose neurons project to the CP/PPn, and neurogenesis in these regions might also influence chirping. The dorsal telencephalon, a region likely homologous to the mammalian hippocampus, likely plays a role in the long-term habituation of chirping behavior (Harvey-Girard et al., 2010). Given the abundant cell proliferation in the teleost telencephalon (Zupanc and Horschke, 1995; Sørensen et al., 2007; von Krogh et al., 2010; Zupanc and Sirbulescu, 2011; Maruska et al., 2012) and the well-documented role of hippocampal neurogenesis to certain forms of learning in mammals (Shors et al., 2001; Marín-Burgin and Schinder, 2012), it is intriguing to ask whether socially induced neurogenesis in the dorsal telencephalon in electric fish might contribute to learned changes in chirping behavior.

List of abbreviations

11-KT	11-ketotestosterone
BrdU	bromodeoxyuridine
CP	central posterior thalamic nucleus
dF	difference frequency
EOD	electric organ discharge
PPn	prepacemaker nucleus
PVZ	periventricular zone

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Author contributions

All authors contributed to the conception, design and data collection for the studies. K.D.D. wrote the majority of the manuscript; M.C. and J.F.C. commented on and revised the manuscript.

Competing interests

No competing interests declared.

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