BIOLOGY LETTERS

royalsocietypublishing.org/journal/rsbl

Research

Cite this article: Ludington SC, McKinney JE, Butler JM, Goolsby BC, Callan AA, Gaines-Richardson M, O'Connell LA. 2024 Activity of forkhead box P2-positive neurons is associated with tadpole begging behaviour. *Biol. Lett.* **20**: 20240395.
https://doi.org/10.1098/rsbl.2024.0395

Received: 9 December 2023
Accepted: 2 September 2024

Subject Category:
Neurobiology

Subject Areas:
behaviour, neuroscience

Keywords:
amphibian, begging, aggression, social behaviour, striatum, cerebellum

Author for correspondence:
Lauren A. O'Connell
e-mail: loconnel@stanford.edu

Electronic supplementary material is available online at https://doi.org/10.6084/m9.figshare.c.7454638.

THE ROYAL SOCIETY PUBLISHING

Activity of forkhead box P2-positive neurons is associated with tadpole begging behaviour

Sarah C. Ludington, Jordan E. McKinney, Julie M. Butler, Billie C. Goolsby, Ashlyn A. Callan, Maiah Gaines-Richardson and Lauren A. O'Connell

Department of Biology, Stanford University, Stanford, CA 94305, USA

JMB, 0000-0002-7400-8780; LAO'C, 0000-0002-2706-4077

Motor function is a critical aspect of social behaviour in a wide range of taxa. The transcription factor forkhead box P2 (FoxP2) is well studied in the context of vocal communication in humans, mice and songbirds, but its role in regulating social behaviour in other vertebrate taxa is unclear. We examined the distribution and activity of FoxP2-positive neurons in tadpoles of the mimic poison frog (*Ranitomeya imitator*). In this species, tadpoles are reared in isolated plant nurseries and are aggressive to other tadpoles. Mothers provide unfertilized egg meals to tadpoles that perform a begging display by vigorously vibrating back and forth. We found that FoxP2 is widely distributed in the tadpole brain and parallels the brain distribution in mammals, birds and fishes. We then tested the hypothesis that FoxP2-positive neurons would have differential activity levels in begging or aggression contexts compared to non-social controls. We found that FoxP2-positive neurons showed increased activation in the striatum and cerebellum during begging and in the nucleus accumbens during aggression. Overall, these findings lay a foundation for testing the hypothesis that FoxP2 has a generalizable role in social behaviour beyond vocal communication across terrestrial vertebrates.

1. Introduction

In species where parents provision their young, offspring signalling can be important for obtaining food. Begging behaviour generally involves coordination of motor circuits, such as vocalization in chicks [1–3], vibrational displays in amphibian tadpoles [4,5] and chemical and motor signals in insect larvae [6,7]. There is a rich theoretical literature on the evolution of offspring signalling and parental investment [8,9], which has been experimentally investigated mostly in birds [10–12]. While the behavioural and physiological ecology of begging has received much attention, the neural basis of this critical behaviour is relatively unknown, with the exception that bird begging behaviour uses the same vocal-motor pathways later used to produce adult songs [13]. Investigating the neural circuits and gene networks that regulate offspring signalling would establish a mechanistic view of how begging behaviour evolves from ancestral neural features. Additionally, this perspective would complement the existing theoretical models of how and when begging signals evolve.

Forkhead box P2 (FoxP2) protein is associated with motor processes related to behaviour in many species. This protein is a transcription factor that regulates gene networks important in many neuronal functions, including genes involved in synaptic plasticity, neurotransmission and axonal guidance [14,15]. Interest in FOXP2 surged when a mutation was linked to speech and language impairments in humans [16] (human FOXP2 and non-human FoxP2

homologues are upper case and lower case, respectively). The mutation of a critical residue in the deoxyribonucleic acid-binding domain of the human FOXP2 (the R553H mutation) causes difficulty with fine, rapid movements of the mouth and face that impair speech [17]. FOXP2 truncations and intragenic deletions also manifest in language and speech impairments [18–20]. Individuals carrying FOXP2 disruptions are at risk for other phenotypes, such as difficulties feeding in infancy and low performance in receptive and expressive language assessments [21]. A conserved role for FoxP2 has been extended to other species, where FoxP2 manipulations influence vocalizations emitted by birds [22–24] and mice [25–27]. Functional studies in humans, mice and birds point to the role of FoxP2 in the development and function of corticostriatal and corticocerebellar circuits important for motor control [16,28,29]. Despite the research emphasis on vocal communication, FoxP2 manipulations in mice lead to altered social interactions, such as parental care and aggression [30,31], as well as skilled motor tasks [32], suggesting a broad role for FoxP2 in coordinating motor aspects of behaviour.

Frogs use both vocal and non-vocal signalling for social interactions [33,34], but the role of FoxP2 in frog communication has not yet been investigated, to our knowledge. Like in mammals [35], FoxP2 is expressed in early brain development of *Xenopus* [36]. Given the expression of FoxP2 in the larval brain and that FoxP2 is important in regulating motor aspects of social behaviour in mammals and birds, we reasoned that FoxP2 may play a role in amphibian social behaviours as well. Specifically, since FoxP2 has an important role in vocal-motor pathways of bird song [22–24], which are more active in begging birds [13], we reasoned that FoxP2 may be involved in tadpole begging behaviour. Additionally, as FoxP2 leads to altered aggression in mice [30], we reasoned that FoxP2 may also be associated with tadpole aggression. In this study, we tested the hypothesis that FoxP2 is associated with begging signals of tadpoles toward adult conspecifics or aggression toward tadpole conspecifics. We tested this hypothesis in the mimic poison frog (*Ranitomeya imitator*), where tadpoles beg parents for unfertilized egg meals by vigorously vibrating back and forth with their heads and nipping at visiting females with their mouths [37]. Tadpoles are reared in isolated nurseries where they are aggressive to intruder tadpoles [4,38]. We first mapped the neural distribution of FoxP2 in the *R. imitator* tadpole brain and then compared the activity of FoxP2-positive neurons across begging, aggressive and control animals. Given the extensive literature of striatal FoxP2 in vocal communication in birds and mice, we predicted that FoxP2-positive neurons in the striatum would have higher activity in begging tadpoles.

2. Methods

(a) Animals

Ranitomeya imitator tadpoles were bred from our laboratory colony [39]. Adult *R. imitator* females from breeding pairs were used as stimulus animals in the begging context. A conspecific tadpole was used as a stimulus in the aggression context. All procedures were approved by the Stanford University Animal Care and Use Committee (protocol no. 33097).

(b) Behaviour

We randomly assigned tadpoles (Gosner stage 30–34, no forelimb development and minimal hindlimb development) into one of three experimental groups: a reproductive adult female (begging, n = 14), a smaller-sized conspecific tadpole (aggression, n = 15) or exposed to a novel object (a metal bolt, n = 15). Conspecific stimuli were different between trials. All behaviour trials were conducted between 09.00 and 12.00 h. Tadpoles were placed into individual square arenas (5 × 5 × 5 cm) filled with 50 ml of conditioned water (Josh's Frogs R/O Rx, Owosso, MI, USA). Tadpoles were recorded from above using GoPro cameras (GoPro HERO7 Black, 1080p, 240 fps). Each tadpole acclimated for 10 min in the arena. Then, the stimulus was introduced to the arena, and behaviour was recorded for 30 min. Stimuli were then removed from the arena, and tadpoles were placed in the dark for 15 min to minimize post-stimulus neural activity. This additional time was included as pilot experiments with pS6-immunoreactivity suggest this marker peaks 45 min post-stimulus. Tadpoles were then anaesthetized with topical 20% benzocaine and euthanized by decapitation.

Videos were scored using BORIS software [40] by an observer uninformed of tadpole identity (electronic supplementary material, figure S1). Begging was quantified by the number and duration of each begging bout, where the tadpole orients to, intensely vibrates near and occasionally nips at a conspecific. Aggression and cannibalism are observed in tadpoles of this species, where tadpoles will attack conspecifics. In this study, aggression was quantified by the number and duration of attacks toward the other tadpole. Control tadpoles did not display either of these behaviours.

(c) Immunohistochemistry

Whole tadpole heads were fixed with 4% paraformaldehyde in 1X phosphate-buffered saline (PBS) at 4°C overnight, rinsed in 1X PBS and transferred to a 30% sucrose solution for cryoprotection at 4°C overnight. Samples were then embedded in mounting media (Tissue-Tek® O.C.T. Compound; Electron Microscopy Sciences, Hatfield, PA, USA) and stored at −80°C until cryosectioning at 15 µm into three series. Sections were thaw-mounted onto SuperFrost Plus microscope slides (VWR International, Radnor, PA, USA) and then stored at −80°C until immunohistochemistry.

We used double-label fluorescence immunohistochemistry to detect FoxP2 and phosphorylated ribosomes (phospho-S6 (pS6)) as a proxy of neural activity [41], as previously described [42]. Slides were incubated overnight in a mix of both primary antibodies (rabbit anti-pS6 (Invitrogen, cat. no. 44-923G) at 1 : 500 and goat anti-FoxP2 (Abcam, cat. no. AB1307) at 1 : 500 in 2%

normal donkey, 0.3% TritonX-100, 1X PBS). Following several washes, slides were incubated in a mix of fluorescent secondary antibodies (1 : 200 Alexa 488 donkey anti-goat and 1 : 2 00 Alexa 568 donkey anti-rabbit in 2% normal donkey serum, 0.3% TritonX-100, 1X PBS) for 2 h. Slides were then rinsed in water and cover-slipped using VECTASHIELD HardSet Mounting Medium with 4′,6-diamidino-2-phenylindole (DAPI; Vector Laboratories, Burlingame, CA, USA) and stored at 4°C. FoxP2 was restricted to cell nuclei, and additional antibody characterization can be found in electronic supplementary material, figures S2–S5.

(d) Fluorescence microscopy and cell counting

Brain sections were imaged on a Leica compound fluorescent microscope with a QImaging Retiga 2000R camera as previously described [42]. Brain regions containing FoxP2 were identified using DAPI-stained nuclei while referencing a poison frog brain atlas [42]. FIJI software [43] was used to measure the area of the nucleus accumbens, striatum and cerebellum within a single hemisphere. The number of FoxP2-positive cells, pS6-positive cells and colocalized cells were quantified within each area using the 'Cell Counter' function. Due to tissue quality, one to four sections were counted per individual per brain region.

(e) Data analysis

All statistics and figures were generated in RStudio (v. 1.1.442) running R (v. 3.5.2). We used the glmmTMB R package [44] to analyse cell count data with generalized linear mixed models. For FoxP2-positive and pS6-FoxP2 colocalized cells, we ran separate models using a negative binomial distribution; model fit was confirmed using DHARMa [45]. For both models, we tested the main effects of the experimental group (begging, aggression and control), brain region and their interaction. Tadpole identity was included as a random variable to account for repeated sampling of brain regions within individuals. The log of the brain region area was included as an offset. For colocalization data, the number of colocalized (pS6 + FoxP2) cells was the dependent variable, and the number of FoxP2 cells was included as a weight in the model. We then used the Anova.glmmTMB function for reported statistical values. When there was a significant interaction between the group and brain region, we ran a *post hoc* test with the emmeans R package (v. 1.5.3) and used false discovery rate correction for multiple hypothesis testing. Correlations between behaviour and cell counts were tested using the cor.test function in the R base package with the Spearman method.

3. Results

(a) Neural distribution of forkhead box P2

We observed a broad distribution of FoxP2-positive cells throughout the tadpole brain (figure 1; electronic supplementary material, figure S6). The highest densities of FoxP2-positive cells were found in the subpallial forebrain, optic tectum, thalamus and cerebellum. Notably, there were many FoxP2 cells in regions linked to sensory processing, such as the olfactory bulb (chemosensory), torus semicircularis (acoustic processing) and optic tectum (vision).

(b) Forkhead box P2-positive neuronal activity changes with different social stimuli

We investigated whether FoxP2-positive neuronal activity is associated with social behaviour by quantifying the proportion of FoxP2-positive cells that colocalized with the pS6 marker of neural activity in tadpoles showing begging or aggression compared to those exposed to a novel object (asocial control; figure 2). We focused our quantification efforts on the basal ganglia (nucleus accumbens and striatum) and cerebellum given their robust expression of FoxP2 in mice and birds [46], and functional studies suggesting FoxP2-associated vocalization deficits are due to altered corticostriatal and corticocerebellar circuits [16,28,29]. The activity of FoxP2-positive cells depended on an interaction of behavioural group and brain region (group × region: $F_4 = 130.66$, $p < 0.001$). Begging tadpoles had more active FoxP2-positive cells than aggressive and control tadpoles in the striatum (Str, aggression vs. begging: $z = -3.144$, $p = 0.005$; begging vs. control: $z = 2.517$, $p = 0.018$) and cerebellum (Cb, aggression vs. begging: $z = -2.490$, $p = 0.019$; begging vs. control: $z = 3.626$, $p < 0.001$). The number of active FoxP2-positive cells did not differ between aggressive and control animals in the striatum ($p = 0.175$) or cerebellum ($p = 0.920$). Aggressive tadpoles had more active FoxP2-positive cells than control tadpoles in the nucleus accumbens (NAcc, $z = 2.989$, $p = 0.008$), whereas the activity of FoxP2-positive cells in this brain region did not differ between begging and aggression ($p = 0.125$) or control ($p = 0.125$) contexts. There was a significant difference in the number of FoxP2-positive cells within these brain regions across groups, where aggressive tadpoles had more FoxP2-positive cells in the striatum (electronic supplementary material, figure S7). There were no significant differences in the number of pS6 cells within these brain regions across groups, and there were no significant correlations between the activity of FoxP2-positive cells and measures of begging or aggressive behaviours (electronic supplementary material, figure S8).

4. Discussion

Across species, there is variation in whether and how young animals display aggression or signalling to caregivers, but both behaviours require motor function coordinated by neural processes. Among other functions, the transcription factor

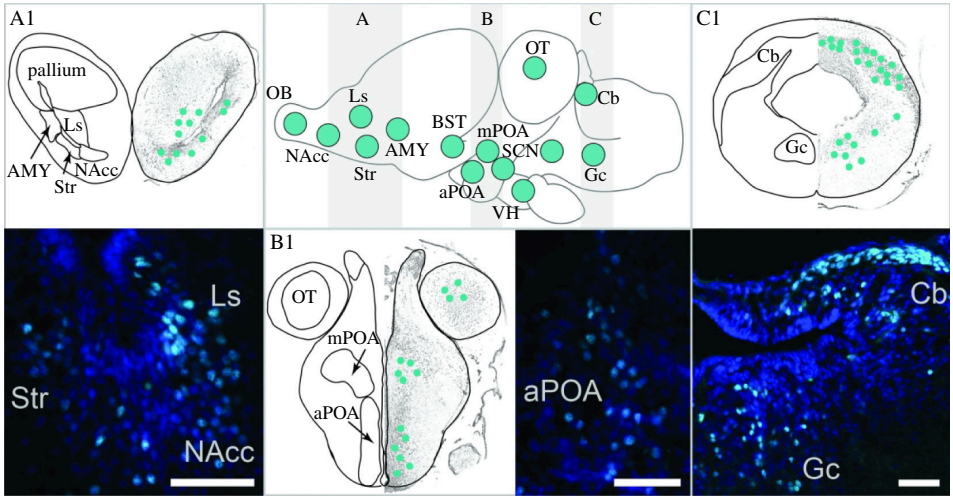

Figure 1. Neural distribution of FoxP2 in amphibians is similar to other vertebrates. FoxP2 is widely distributed throughout the amphibian brain, including the subpallial forebrain (A), midbrain (B) and a few hindbrain regions (C). The centre sagittal brain schematic (rostral is to the left) shows brain regions (green) with FoxP2-positive cells. Grey boxes represent areas of interest for more detailed neuroanatomy and micrographs (A1–C1), where green dots represent the qualitative presence of FoxP2. Micrographs show FoxP2-positive cells (cyan) and DAPI-stained nuclei (blue). Scale bar, 20 µm. The complete neural distribution for FoxP2 can be found in the electronic supplementary material. Abbreviations: AMY, amygdala; aPOA, anterior preoptic area; BST, bed nucleus of the stria terminalis; Cb, cerebellum; Gc, central grey; Ls, lateral septum; mPOA, magnocellular preoptic area; NAcc, nucleus accumbens; OB, olfactory bulb; OT, optic tectum; SCN, suprachiasmatic nucleus; Str, striatum; VH, ventral hypothalamus.

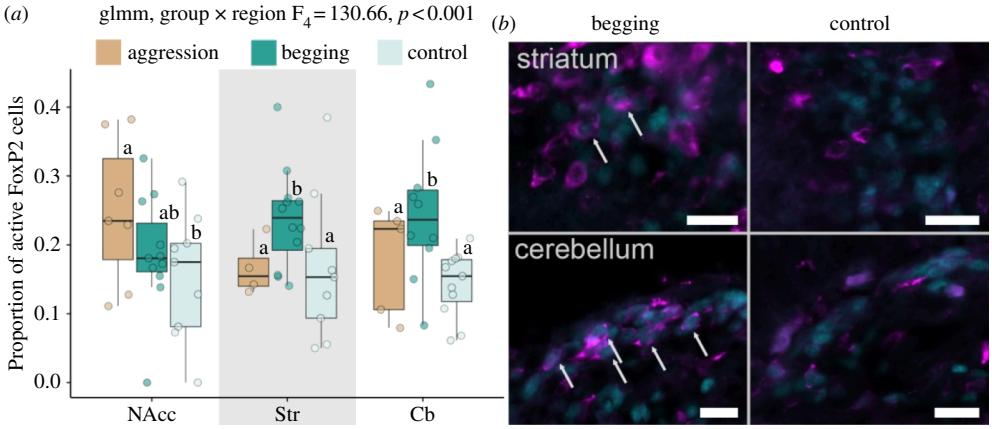

Figure 2. Activity of FoxP2 neurons changes with social behaviour. (*a*) Proportion of active FoxP2-positive cells in aggressive (orange), begging (dark green) and control (light green) tadpoles are shown in boxplots with individual tadpoles displayed with dots. Within each brain region, groups not connected by the same letter are significantly different. (*b*) Representative micrographs of FoxP2 (green) and pS6 (pink) colocalization in the striatum (top) and cerebellum (bottom) of begging (left) or control (right) tadpoles. Scale bar, 10 µm. Abbreviations: Cb, cerebellum; NAcc, nucleus accumbens; Str, striatum.

FoxP2 plays a well-established role in coordinating social behaviours, such as vocal communication in mammals and birds [17,20,24,28,47,48]. Our study expands the role of FoxP2 to social behaviour in amphibians, laying a foundation for testing the generalizable function of FoxP2 in coordinating aspects of social behaviour across terrestrial vertebrates in future studies.

(a) The brain distribution of forkhead box P2 is conserved across vertebrates

FoxP2 is widespread throughout the amphibian brain, with a distribution pattern consistent with those found in other vertebrates (mammals: [35,49,50], birds: [51] and fish: [52–54]). Across these taxa, there is a conserved pattern of expression in brain areas involved in motor output, sensory processing and sensorimotor integration. In *R. imitator* tadpoles, brain regions that regulate motor output and social behaviour, including the basal ganglia and cerebellum, had many FoxP2-positive cells. FoxP2 is expressed in the basal ganglia and cerebellum in avian vocal and non-vocal learners, crocodiles and rodents, suggesting conserved expression in motor-related areas regardless of the ability to learn acoustic communication [49,51]. Although there is conservation in the presence of FoxP2, its abundance is variable in songbirds depending on age and environment, suggesting FoxP2 expression may be linked to periods of vocal plasticity [51]. We also noted FoxP2-positive cells in many sensory processing regions such as the olfactory bulb (chemosensory), optic tectum (visual processing) and torus semicircularis (acoustic processing). In bats, species differences in FoxP2 expression in the olfactory bulb are associated with different feeding habits (frugivorous vs. insectivorous) [55], suggesting that FoxP2 may influence olfactory processing. Given *R. imitator* tadpoles rely on smell to distinguish between heterospecific stimuli [39], investigating FoxP2's role in sensory integration broadly may be a valuable future research direction. However, the expression pattern of FoxP2 is variable across sex, age and species [49,56],

including species differences in the expression of FoxP2 in neuronal and non-neuronal cells in the mammalian cortex [57]. This variability in expression makes the regulatory role of FoxP2 in behaviour unclear but a fruitful avenue of future research. Overall, the distribution of FoxP2 in the amphibian brain suggests a largely conserved pattern across terrestrial vertebrates.

(b) A general role for forkhead box P2 in social behaviour

We found that activity of FoxP2-positive neurons was higher in the striatum and cerebellum of begging tadpoles and in the nucleus accumbens of aggressive tadpoles. Whether this pattern is directly relevant to these behaviours requires functional manipulations in a brain region-specific manner. Regardless, the context-dependent neuronal activation points to brain region-specific roles for FoxP2-positive cells in social behaviour. These findings lay a foundation for testing the hypothesis that FoxP2 has a generalizable role in social behaviour beyond vocal communication.

The striatum is important for motor skills in many vertebrates [58] and has been linked to vocal communication in several taxa [59]. We found that FoxP2-positive cells in the striatum have increased activity during tadpole begging, suggesting a function for this brain region in tadpole signalling. This is supported by many studies regarding the role of FoxP2 in the striatum of vocalizing birds and mammals. Deficits in songbird vocalizations are observed after FoxP2 knockdown in Area X, a striatal nucleus involved in song learning [24]. At a cellular level, FoxP2 has been implicated in structural plasticity, where FoxP2 modifications influence spiny dynamics of Area X neurons in zebra finches [60] and dendrite lengths of striatal neurons in mice [61]. In this same study, the variant of FoxP2 expressed in these mice also impacted dopamine concentrations in the striatum and nucleus accumbens. Dopamine signalling is critical to tadpole begging behaviour [39], and our results here suggest a potential role for FoxP2 in dopamine signalling that should be investigated in the future. This general cellular dysregulation can be seen in mice with FoxP2 mutations, where the striatum is more active and motor-skill learning is disrupted due to abnormal temporal coordination of striatal firing [62]. Together, our work expands the potential role of FoxP2 in the striatum to behavioural signalling in amphibians, suggesting a conserved role for striatal FoxP2 in communication across tetrapod vertebrates.

The cerebellum is a highly conserved vertebrate brain region that coordinates voluntary movements and motor learning [63]. The cerebellum is also implicated in language [64], as there is higher overall cerebellar activity during language tasks in humans [65]. Mice expressing the FoxP2 with the R552H mutation (that leads to speech–language disorders in humans) have impaired ultrasonic vocalizations and poor dendritic development of FoxP2-positive cerebellar Purkinje cells [46]. A reduction of FoxP2 expression, specifically in cerebellar Purkinje neurons, leads to a reduction of ultrasonic vocalizations in mouse pups [66]. Moreover, expressing the wild-type human FOXP2 in the cerebellum partially rescues ultrasonic vocalizations in mice with global expression of FoxP2 with the R552H mutation [67]. To our knowledge, the role of FoxP2 in the cerebellum during vocal learning in songbirds is unknown, but cerebellar lesions impair song learning [68]. Our study, along with studies in neonatal mice, suggests that investigating the function of cerebellar FoxP2 during vocal signalling in songbirds would resolve whether the role of these neurons in coordinating motor signalling is generalizable across taxa.

The nucleus accumbens is involved in motivation and behavioural reinforcement. In this study, we found increased colocalization of pS6 and FoxP2 in the nucleus accumbens of aggressive tadpoles compared to controls. In mice, increased neural activation in the nucleus accumbens is observed with aggression-seeking behaviour [69]. Only one study, to our knowledge, has examined the role of FoxP2 specifically in the nucleus accumbens, where deletion in adult mice leads to altered reward and fear learning [70]. This study did not report effects on aggression. Heterozygous $FoxP2^{+/-}$ mice show altered aggression in resident–intruder and maternal aggression assays, although the brain regions regulating these altered behaviours were not studied [31]. Our data suggest that investigating the nucleus accumbens FoxP2 function in the context of aggression would be a fruitful avenue of research.

5. Summary

We present evidence that FoxP2 has conserved brain expression patterns across vertebrates by filling a critical taxonomic gap from amphibians. We also show that parent-directed signalling by tadpoles is associated with the activity of FoxP2-positive cells in the striatum and cerebellum. In contrast, the activity of FoxP2-positive cells in the nucleus accumbens was associated with aggression. Overall, this work supports the hypothesis that the FoxP2 transcription factor is part of a molecular toolkit important for social behaviour via striatal and cerebellar circuits across many animals.

Ethics. All animal procedures were approved by the Stanford University Animal Care and Use Committee (protocol no. 33097).

Data accessibility. All data are included in electronic supplementary material [71].

Declaration of AI use. We have not used AI-assisted technologies in creating this article.

Authors' contributions. S.C.L.: conceptualization, data curation, formal analysis, investigation, methodology, visualization, writing—original draft; J.E.M.: investigation, writing—review and editing; J.M.B.: investigation, supervision, writing—review and editing; B.C.G.: investigation, visualization, writing—review and editing; A.A.C.: investigation, visualization, writing—review and editing; M.G.-R.: investigation, writing—review and editing; L.A.O.: conceptualization, formal analysis, funding acquisition, project administration, resources, supervision, writing—original draft.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

Funding. This work was supported by the National Institutes of Health (DP2HD102042), the Rita Allen Foundation, Pew Charitable Trusts and The New York Stem Cell Foundation to L.A.O. L.A.O. is a New York Stem Cell Foundation—Robertson Investigator. S.C.L. and J.E.M. were supported by a Stanford University Biology Summer Undergraduate Research Program Fellowship and S.C.L. was supported by a Stanford University Major Grant.

Acknowledgements. We thank Madison Lacey and David Ramirez for their support maintaining our poison frog colony. We especially appreciate comments on early versions of this manuscript from Neil Khosla. We acknowledge that our work takes place on the ancestral and unceded land of the Muwekma Ohlone Tribe.

References

1. Rosenblatt JS. 2003 Outline of the evolution of behavioral and nonbehavioral patterns of parental care among the vertebrates: critical characteristics of mammalian and avian parental behavior. *Scand. J. Psychol.* **44**, 265–271. (doi:10.1111/1467-9450.00344)
2. Mock DW, Dugas MB, Strickler SA. 2011 Honest begging: expanding from signal of need. *Behav. Ecol.* **22**, 909–917. (doi:10.1093/beheco/arr091)
3. Budden AE, Wright J. 2001 Begging in nestling birds. In *Current ornithology*, pp. 83–118, vol. **16**. Boston, MA: Springer US. (doi:10.1007/978-1-4615-1211-0_2)
4. Yoshioka M, Meeks C, Summers K. 2016 Evidence for begging as an honest signal of offspring need in the biparental mimic poison frog. *Anim. Behav.* **113**, 1–11. (doi:10.1016/j.anbehav.2015.12.024)
5. Stynoski JL, Stynoski PB, Noble VR. 2018 Empirical evidence for multiple costs of begging in poison frog tadpoles. *Zool. Anz.* **273**, 203–209. (doi:10.1016/j.jcz.2018.01.012)
6. Smiseth PT, Moore AJ. 2007 Signalling of hunger by senior and junior larvae in asynchronous broods of a burying beetle. *Anim. Behav.* **74**, 699–705. (doi:10.1016/j.anbehav.2006.09.022)
7. Creemers B, Billen J, Gobin B. 2003 Larval begging behaviour in the ant myrmica rubra. *Ethol. Ecol. Evol.* **15**, 261–272. (doi:10.1080/08927014.2003.9522671)
8. Trivers RL. 1974 Parent-offspring conflict. *Am. Zool.* **14**, 249–264. (doi:10.1093/icb/14.1.249)
9. Godfray HC. 1995 Evolutionary theory of parent-offspring conflict. *Nature* **376**, 133–138. (doi:10.1038/376133a0)
10. Hinde CA, Johnstone RA, Kilner RM. 2010 Parent–offspring conflict and coadaptation. *Science* **327**, 1373–1376. (doi:10.1126/science.1186056)
11. Dearborn DC. 1998 Begging behavior and food acquisition by brown-headed cowbird nestlings. *Behav. Ecol. Sociobiol.* **43**, 259–270. (doi:10.1007/s002650050490)
12. McCarty JP. 1996 The energetic cost of begging in nestling passerines. *Auk* **113**, 178–188. (doi:10.2307/4088944)
13. Liu WC, Rivers JW, White DJ. 2016 Vocal matching and intensity of begging calls are associated with a forebrain song circuit in a generalist brood parasite. *Dev. Neurobiol.* **76**, 615–625. (doi:10.1002/dneu.22348)
14. Vernes SC, Spiteri E, Nicod J, Groszer M, Taylor JM, Davies KE, Geschwind DH, Fisher SE. 2007 High-throughput analysis of promoter occupancy reveals direct neural targets of *FOXP2*, a gene mutated in speech and language disorders. *Am. J. Hum. Genet.* **81**, 1232–1250. (doi:10.1086/522238)
15. Vernes SC *et al*. 2011 Foxp2 regulates gene networks implicated in neurite outgrowth in the developing brain. *PLoS Genet.* **7**, e1002145. (doi:10.1371/journal.pgen.1002145)
16. Vargha-Khadem F, Gadian DG, Copp A, Mishkin M. 2005 *FOXP2* and the neuroanatomy of speech and language. *Nat. Rev. Neurosci.* **6**, 131–138. (doi:10.1038/nrn1605)
17. Lai CSL, Fisher SE, Hurst JA, Vargha-Khadem F, Monaco AP. 2001 A forkhead-domain gene is mutated in a severe speech and language disorder. *Nature* **413**, 519–523. (doi:10.1038/35097076)
18. Reuter MS *et al*. 2017 *FOXP2* variants in 14 individuals with developmental speech and language disorders broaden the mutational and clinical spectrum. *J. Med. Genet.* **54**, 64–72. (doi:10.1136/jmedgenet-2016-104094)
19. MacDermot KD *et al*. 2005 Identification of FOXP2 truncation as a novel cause of developmental speech and language deficits. *Am. J. Hum. Genet.* **76**, 1074–1080. (doi:10.1086/430841)
20. Turner SJ, Hildebrand MS, Block S, Damiano J, Fahey M, Reilly S, Bahlo M, Scheffer IE, Morgan AT. 2013 Small intragenic deletion in *FOXP2* associated with childhood apraxia of speech and dysarthria. *Am. J. Med. Genet. A* **161A**, 2321–2326. (doi:10.1002/ajmg.a.36055)
21. Morison LD *et al*. 2023 In-depth characterisation of a cohort of individuals with missense and loss-of-function variants disrupting. *J. Med. Genet.* **60**, 597–607. (doi:10.1136/jmg-2022-108734)
22. Day NF, Hobbs TG, Heston JB, White SA. 2019 Beyond critical period learning: striatal FOXP2 affects the active maintenance of learned vocalizations in adulthood. *eNeuro* **6**. (doi:10.1523/ENEURO.0071-19.2019)
23. Murugan M, Harward S, Scharff C, Mooney R. 2013 Diminished FoxP2 levels affect dopaminergic modulation of corticostriatal signaling important to song variability. *Neuron* **80**, 1464–1476. (doi:10.1016/j.neuron.2013.09.021)
24. Haesler S, Rochefort C, Georgi B, Licznerski P, Osten P, Scharff C. 2007 Incomplete and inaccurate vocal imitation after knockdown of FoxP2 in songbird basal ganglia nucleus area X. *PLoS Biol.* **5**, e321. (doi:10.1371/journal.pbio.0050321)
25. Chabout J, Sarkar A, Patel SR, Radden T, Dunson DB, Fisher SE, Jarvis ED. 2016 A Foxp2 mutation implicated in human speech deficits alters sequencing of ultrasonic vocalizations in adult male mice. *Front. Behav. Neurosci.* **10**, 197. (doi:10.3389/fnbeh.2016.00197)
26. Castellucci GA, McGinley MJ, McCormick DA. 2016 Knockout of Foxp2 disrupts vocal development in mice. *Sci. Rep.* **6**, 23305. (doi:10.1038/srep23305)
27. Shu W *et al*. 2005 Altered ultrasonic vocalization in mice with a disruption in the Foxp2 gene. *Proc. Natl Acad. Sci. USA* **102**, 9643–9648. (doi:10.1073/pnas.0503739102)
28. Lai CSL, Gerrelli D, Monaco AP, Fisher SE, Copp AJ. 2003 *FOXP2* expression during brain development coincides with adult sites of pathology in a severe speech and language disorder. *Brain* **126**, 2455–2462. (doi:10.1093/brain/awg247)
29. Teramitsu I, Kudo LC, London SE, Geschwind DH, White SA. 2004 Parallel *FoxP1* and *FoxP2* expression in songbird and human brain predicts functional interaction. *J. Neurosci.* **24**, 3152–3163. (doi:10.1523/JNEUROSCI.5589-03.2004)
30. Medvedeva VP *et al*. 2019 Altered social behavior in mice carrying a cortical *Foxp2* deletion. *Hum. Mol. Genet.* **28**, 701–717. (doi:10.1093/hmg/ddy372)
31. Herrero MJ, Wang L, Hernandez-Pineda D, Banerjee P, Matos HY, Goodrich M, Panigrahi A, Smith NA, Corbin JG. 2021 Sex-specific social behavior and amygdala proteomic deficits in mutant mice. *Front. Behav. Neurosci.* **15**, 706079. (doi:10.3389/fnbeh.2021.706079)
32. French CA, Vinueza Veloz MF, Zhou K, Peter S, Fisher SE, Costa RM, De Zeeuw CI. 2019 Differential effects of Foxp2 disruption in distinct motor circuits. *Mol. Psychiatry* **24**, 447–462. (doi:10.1038/s41380-018-0199-x)
33. Narins PM, Feng AS, Fay RR. 2006 *Hearing and sound communication in amphibians*. New York, NY: Springer. (doi:10.1007/978-0-387-47796-1)

34. Kelley DB. 2004 Vocal communication in frogs. *Curr. Opin. Neurobiol.* **14**, 751–757. (doi:10.1016/j.conb.2004.10.015)
35. Ferland RJ, Cherry TJ, Preware PO, Morrisey EE, Walsh CA. 2003 Characterization of Foxp2 and Foxp1 mRNA and protein in the developing and mature brain. *J. Comp. Neurol.* **460**, 266–279. (doi:10.1002/cne.10654)
36. Schön C, Wochnik A, Rössner A, Donow C, Knöchel W. 2006 The FoxP subclass in *Xenopus laevis* development. *Dev. Genes Evol.* **216**, 641–646. (doi:10.1007/s00427-006-0073-8)
37. Brown JL, Morales V, Summers K. 2010 A key ecological trait drove the evolution of biparental care and monogamy in an amphibian. *Am. Nat.* **175**, 436–446. (doi:10.1086/650727)
38. McKinney JE, Ludington SC, Butler JM, O'Connell LA. 2022 Proopiomelanocortin (POMC) is a negative regulator of tadpole aggression through opioid receptor signaling. *bioRxiv*. (doi:10.1101/2022.11.28.518266)
39. Butler JM, Singh D, Baker P, Edwards SV, Summers K, O'Connell LA. 2023 Dopamine neurons govern olfactory-gated infant begging behavior. *bioRxiv*. (doi:10.1101/2023.03.18.533277)
40. Friard O, Gamba M. 2016 BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. *Methods Ecol. Evol.* **7**, 1325–1330. (doi:10.1111/2041-210X.12584)
41. Knight ZA, Tan K, Birsoy K, Schmidt S, Garrison JL, Wysocki RW, Emiliano A, Ekstrand MI, Friedman JM. 2012 Molecular profiling of activated neurons by phosphorylated ribosome capture. *Cell* **151**, 1126–1137. (doi:10.1016/j.cell.2012.10.039)
42. Fischer EK, Roland AB, Moskowitz NA, Tapia EE, Summers K, Coloma LA, O'Connell LA. 2019 The neural basis of tadpole transport in poison frogs. *Proc. R. Soc. B* **286**, 20191084. (doi:10.1098/rspb.2019.1084)
43. Schindelin J *et al*. 2012 Fiji: an open-source platform for biological-image analysis. *Nat. Methods* **9**, 676–682. (doi:10.1038/nmeth.2019)
44. Brooks M Kristensen K, Benthem K Magnusson A, Berg C Nielsen A, Skaug H Mächler M, Bolker B 2017 glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *R J.* **9**, 378. (doi:10.32614/RJ-2017-066)
45. Hartig F. DHARMa: residual diagnostics for hierarchical (multi-level/mixed) regression models. R package version 0.3. See https://CRAN.R-project.org/package=DHARMa.
46. Fujita E, Tanabe Y, Shiota A, Ueda M, Suwa K, Momoi MY, Momoi T. 2008 Ultrasonic vocalization impairment of Foxp2 (R552H) knockin mice related to speech-language disorder and abnormality of Purkinje cells. *Proc. Natl Acad. Sci. USA* **105**, 3117–3122. (doi:10.1073/pnas.0712298105)
47. Callaway E. 2011 'Language gene' speeds learning. *Nature* (doi:10.1038/nature.2011.9395)
48. Bowers JM, Perez-Pouchoulen M, Edwards NS, McCarthy MM. 2013 Foxp2 mediates sex differences in ultrasonic vocalization by rat pups and directs order of maternal retrieval. *J. Neurosci.* **33**, 3276–3283. (doi:10.1523/JNEUROSCI.0425-12.2013)
49. Campbell P, Reep RL, Stoll ML, Ophir AG, Phelps SM. 2009 Conservation and diversity of Foxp2 expression in muroid rodents: functional implications. *J. Comp. Neurol.* **512**, 84–100. (doi:10.1002/cne.21881)
50. Rodenas-Cuadrado PM, Mengede J, Baas L, Devanna P, Schmid TA, Yartsev M, Firzlaff U, Vernes SC. 2018 Mapping the distribution of language related genes *FoxP1*, *FoxP2*, and *CntnaP2* in the brains of vocal learning bat species. *J. Comp. Neurol.* **526**, 1235–1266. (doi:10.1002/cne.24385)
51. Haesler S, Wada K, Nshdejan A, Morrisey EE, Lints T, Jarvis ED, Scharff C. 2004 *FoxP2* expression in avian vocal learners and non-learners. *J. Neurosci.* **24**, 3164–3175. (doi:10.1523/JNEUROSCI.4369-03.2004)
52. Shah R, Medina-Martinez O, Chu LF, Samaco RC, Jamrich M. 2006 Expression of *FoxP2* during zebrafish development and in the adult brain. *Int. J. Dev. Biol.* **50**, 435–438. (doi:10.1387/ijdb.052065rs)
53. Itakura T *et al*. 2008 The medaka FoxP2, a homologue of human language gene *FOXP2*, has a diverged structure and function. *J. Biochem.* **143**, 407–416. (doi:10.1093/jb/mvm235)
54. Pengra IGG, Marchaterre MA, Bass AH. 2018 FoxP2 expression in a highly vocal teleost fish with comparisons to tetrapods. *Brain Behav. Evol.* **91**, 82–96. (doi:10.1159/000487793)
55. Chen Q, Wang L, Jones G, Metzner W, Xuan FJ, Yin J, Sun Y. 2013 *FoxP2* and olfaction: divergence of *FoxP2* expression in olfactory tubercle between different feeding habit bats. *Acta. Biol. Hung.* **64**, 426–437. (doi:10.1556/ABiol.64.2013.4.3)
56. Thompson CK, Schwabe F, Schoof A, Mendoza E, Gampe J, Rochefort C, Scharff C. 2013 Young and intense: FoxP2 immunoreactivity in area X varies with age, song stereotypy, and singing in male zebra finches. *Front. Neural Circuits* **7**, 24. (doi:10.3389/fncir.2013.00024)
57. Ma S *et al*. 2022 Molecular and cellular evolution of the primate dorsolateral prefrontal cortex. *Science* **377**, eabo7257. (doi:10.1126/science.abo7257)
58. Cataldi S, Stanley AT, Miniaci MC, Sulzer D. 2022 Interpreting the role of the striatum during multiple phases of motor learning. *FEBS J.* **289**, 2263–2281. (doi:10.1111/febs.15908)
59. Konopka G, Roberts TF. 2016 Animal models of speech and vocal communication deficits associated with psychiatric disorders. *Biol. Psychiatry* **79**, 53–61. (doi:10.1016/j.biopsych.2015.07.001)
60. Schulz SB, Haesler S, Scharff C, Rochefort C. 2010 Knockdown of FoxP2 alters spine density in area X of the zebra finch. *Genes Brain Behav.* **9**, 732–740. (doi:10.1111/j.1601-183X.2010.00607.x)
61. Enard W *et al*. 2009 A humanized version of Foxp2 affects cortico-basal ganglia circuits in mice. *Cell* **137**, 961–971. (doi:10.1016/j.cell.2009.03.041)
62. French CA, Jin X, Campbell TG, Gerfen E, Groszer M, Fisher SE, Costa RM. 2012 An aetiological *Foxp2* mutation causes aberrant striatal activity and alters plasticity during skill learning. *Mol. Psychiatry* **17**, 1077–1085. (doi:10.1038/mp.2011.105)
63. Sokolov AA, Miall RC, Ivry RB. 2017 The cerebellum: adaptive prediction for movement and cognition. *Trends Cogn. Sci.* **21**, 313–332. (doi:10.1016/j.tics.2017.02.005)
64. Mariën P, Borgatti R. 2018 Language and the cerebellum. *Handb. Clin. Neurol.* **154**, 181–202. (doi:10.1016/B978-0-444-63956-1.00011-4)
65. E K-H, Chen S-HA, Ho M-HR, Desmond JE. 2014 A meta-analysis of cerebellar contributions to higher cognition from PET and fMRI studies. *Hum. Brain Mapp.* **35**, 593–615. (doi:10.1002/hbm.22194)
66. Usui N, Co M, Harper M, Rieger MA, Dougherty JD, Konopka G. 2017 Sumoylation of FOXP2 regulates motor function and vocal communication through Purkinje cell development. *Biol. Psychiatry* **81**, 220–230. (doi:10.1016/j.biopsych.2016.02.008)
67. Fujita-Jimbo E, Momoi T. 2014 Specific expression of FOXP2 in cerebellum improves ultrasonic vocalization in heterozygous but not in homozygous *Foxp2* (R552H) knock-in pups. *Neurosci. Lett.* **566**, 162–166. (doi:10.1016/j.neulet.2014.02.062)
68. Pidoux L, Le Blanc P, Levenes C, Leblois A. 2018 A subcortical circuit linking the cerebellum to the basal ganglia engaged in vocal learning. *eLife* **7**, e32167. (doi:10.7554/eLife.32167)
69. Golden SA, Jin M, Heins C, Venniro M, Michaelides M, Shaham Y. 2019 Nucleus accumbens Drd1-expressing neurons control aggression self-administration and aggression seeking in mice. *J. Neurosci.* **39**, 2482–2496. (doi:10.1523/JNEUROSCI.2409-18.2019)
70. He BH, Yang YH, Hsiao BW, Lin WT, Chuang YF, Chen SY, Liu FC. 2024 *Foxp2* is required for nucleus accumbens-mediated multifaceted limbic function. *Neuroscience* **542**, 33–46. (doi:10.1016/j.neuroscience.2024.02.004)
71. Ludington SC, McKinney JE, Butler JM, Goolsby BC, Callan AA, Gaines-Richardson M, O'Connell LA. 2024 Data from: Activity of FoxP2-positive neurons is associated with tadpole begging behavior. Figshare. (doi:10.6084/m9.figshare.c.7454638)