



Risky Ripples Allow Bats and Frogs to Eavesdrop on a Multisensory Sexual Display

W. Halfwerk *et al.*

Science **343**, 413 (2014);

DOI: 10.1126/science.1244812

This copy is for your personal, non-commercial use only.

If you wish to distribute this article to others, you can order high-quality copies for your colleagues, clients, or customers by [clicking here](#).

Permission to republish or repurpose articles or portions of articles can be obtained by following the guidelines [here](#).

The following resources related to this article are available online at www.sciencemag.org (this information is current as of February 3, 2014):

Updated information and services, including high-resolution figures, can be found in the online version of this article at:

<http://www.sciencemag.org/content/343/6169/413.full.html>

Supporting Online Material can be found at:

<http://www.sciencemag.org/content/suppl/2014/01/23/343.6169.413.DC1.html>

This article **cites 27 articles**, 7 of which can be accessed free:

<http://www.sciencemag.org/content/343/6169/413.full.html#ref-list-1>

This article appears in the following **subject collections**:

Ecology

<http://www.sciencemag.org/cgi/collection/ecology>

sensitivities of the photoreceptors in *H. trispinosa* were measured using intracellular electrophysiology (9) (table S1). Eight sensitivity maxima were found in the visible part of the spectrum, and another three were found in the UV {the fourth UV cell often proving hard to locate and record from [(9), Fig. 1]}. We modeled the stomatopod spectral discrimination curve using the Vorobyev/Osorio noise-limited model (21) (which determines color thresholds using photoreceptor noise levels) for a serial dichromatic system with comparison between each adjacent spectral sensitivity [mechanism (i) above (note S1)]. This system predicts very fine discrimination between 1 to 5 nm throughout the spectrum, with few peaks of coarser discrimination as seen in other animals. Such fine spectral discrimination would be expected in a color vision system that made analog comparisons between adjacent spectral sensitivities. The behavioral results presented here (Fig. 2) suggest that such analog comparisons are not made. Instead, stomatopod color vision is remarkably coarse (Fig. 3).

The results from our experiments suggest that the stomatopods do not use a processing system of multiple dichromatic comparisons as previously hypothesized based on assumed neural connections (16). Instead, we provide evidence that scanning eye movements (22) may generate a temporal signal for each spectral sensitivity, enabling them to recognize colors instead of discriminating them. (Fig. 4) (3, 4). In such a system, the 12 sensitivities (including the UV, not analyzed here but with its multiple sensitivities a good fit to the system envisaged) would be converted into a temporal pattern when scanned across an object, which the animal could recognize as color. This system is comparable to the spectral linear analyzers (termed "push-broom" analyzers because of the arrangement of the sensors and the flight direction) used in remote sensing systems (23) and is a unique way for animals to encode color. Although this system does not have the ability to discriminate between closely positioned wavelengths (and results in spectral "discrimination" defined by the distances between sensitivity peaks, seen when comparing Fig. 1 and 3), it would enable the stomatopod to make quick and reliable determinations of color, without the processing delay required for a multidimensional color space. Without the comparison of spectral channels, color constancy would not function in the way we currently understand it in other animals. Instead, identification of a color pattern by the mid-band and luminance by the hemispheres might function as a "panchromatic" method to discount illuminance (23). The eye is optically skewed so that both midband and hemispheres examine the same areas in space, which lends support to this idea (3). However, the details of the neural processing from the receptors remain unknown.

Stomatopods live a rapid-fire lifestyle of combat and territoriality, so possessing a simple, temporally efficient color recognition ability may be critical for survival (24, 25). As with many invertebrate information-encoding solutions, the actual processing of the problem is dealt with at

the periphery, in this case by an array of detectors seen in animals and unconsciously duplicated by remote-sensing engineers. What remains for us to discover is the nature of the information and its importance in the biological decisions these engaging crustaceans make.

References and Notes

- N. J. Marshall, *Nature* **333**, 557–560 (1988).
- T. Cronin, N. J. Marshall, *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.* **166**, 261 (1989).
- J. Marshall, T. W. Cronin, S. Kleinlogel, *Arthropod Struct. Dev.* **36**, 420–448 (2007).
- C. Neumeyer, in *Evolution of the Eye and Visual System*, J. R. Cronly-Dillon, Ed. (Macmillan, London, 1991), vol. 2, pp. 284–305.
- N. J. Marshall, J. P. Jones, T. W. Cronin, *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.* **179**, 473 (1996).
- S. Kleinlogel, N. J. Marshall, J. M. Horwood, M. F. Land, *J. Comp. Neurol.* **467**, 326–342 (2003).
- K. R. Gegenfurtner, D. C. Kiper, *Annu. Rev. Neurosci.* **26**, 181–206 (2003).
- J. Mollon, C. Cavonius, in *Colour Vision Deficiencies VIII*, G. Verriest, Ed. (Springer, Netherlands, 1986), pp. 473–483.
- J. Marshall, J. Oberwinkler, *Nature* **401**, 873–874 (1999).
- T.-H. Chiou *et al.*, *Curr. Biol.* **18**, 429–434 (2008).
- K. Arikawa, *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.* **189**, 791–800 (2003).
- H. B. Barlow, *Vision Res.* **22**, 635–643 (1982).
- L. T. Maloney, *J. Opt. Soc. Am. A* **3**, 1673–1683 (1986).
- M. Vorobyev, in *Biophysics of Photoreception: Molecular and Phototransductive Events*, C. Tadei-Ferreti, Ed. (World Scientific, Singapore, 1997), pp. 263–272.
- A. Kelber, M. Vorobyev, D. Osorio, *Biol. Rev. Camb. Philos. Soc.* **78**, 81–118 (2003).

- N. J. Marshall, M. F. Land, C. A. King, T. W. Cronin, *Philos. Trans. R. Soc. London Ser. B* **334**, 57–84 (1991).
- C. H. Mazel, T. W. Cronin, R. L. Caldwell, N. J. Marshall, *Science* **303**, 51 (2004).
- E. Adams, R. Caldwell, *Anim. Behav.* **39**, 706–716 (1990).
- D. Osorio, N. J. Marshall, T. W. Cronin, *Vision Res.* **37**, 3299–3309 (1997).
- H. Koshitaka, M. Kinoshita, M. Vorobyev, K. Arikawa, *Proc. R. Soc. B Biol. Sci.* **275**, 947–954 (2008).
- M. Vorobyev, D. Osorio, *Proc. R. Soc. B Biol. Sci.* **265**, 351–358 (1998).
- M. F. Land, J. N. Marshall, D. Brownless, T. W. Cronin, *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.* **167**, 155 (1990).
- H. D. Wolpert, *Opt. Photon.* **22**, 16–19 (2011).
- H. Dingle, R. L. Caldwell, *Behaviour* **33**, 115–136 (1969).
- R. L. Caldwell, H. Dingle, *Naturwissenschaften* **62**, 214–222 (1975).

Acknowledgments: This work was supported by grants from the Asian Office of Aerospace Research and Development, the Air Force Office of Scientific Research, the Australian Research Council, the Lizard Island Research Foundation, and a Doctoral Fellowship (2013) from the Lizard Island Research Station, a facility of the Australian Museum. We thank the reviewers for their great comments and W.-S. Chung, M. Bue, and R. Bedford for technical help. All data described in this manuscript are presented in either the main manuscript or the supplementary materials.

Supplementary Materials

www.sciencemag.org/content/343/6169/411/suppl/DC1
Materials and Methods
Figs. S1 and S2
Tables S1 to S3
References (26–29)

11 September 2013; accepted 19 November 2013
10.1126/science.1245824

Risky Ripples Allow Bats and Frogs to Eavesdrop on a Multisensory Sexual Display

W. Halfwerk,^{1, 2*} P. L. Jones,^{1, 3} R. C. Taylor,⁴ M. J. Ryan,^{1, 3} R. A. Page¹

Animal displays are often perceived by intended and unintended receivers in more than one sensory system. In addition, cues that are an incidental consequence of signal production can also be perceived by different receivers, even when the receivers use different sensory systems to perceive them. Here we show that the vocal responses of male túngara frogs (*Physalaemus pustulosus*) increase twofold when call-induced water ripples are added to the acoustic component of a rival's call. Hunting bats (*Trachops cirrhosus*) can echolocate this signal by-product and prefer to attack model frogs when ripples are added to the acoustic component of the call. This study illustrates how the perception of a signal by-product by intended and unintended receivers through different sensory systems generates both costs and benefits for the signaler.

Elaborate courtship displays are favored by sexual selection but are often opposed by predation (1, 2). Animals can produce complex signals that are detected and processed through multiple sensory systems [commonly referred to as multimodal or multisensory signals (3, 4)]. Many communication systems once thought to operate primarily in a single sensory mode actually include secondary components that stimulate additional

senses (5–7). For instance, lip movements are necessary to produce human speech, but the associated visual cue of moving lips can also have a dramatic impact on speech perception (7). Such secondary signal components can be beneficial when they enhance the detection and perception of signals (3, 4). However, communication between the sender and intended receiver rarely occurs in private channels (8), and signalers are prone to costs imposed by eavesdroppers, such as predators and parasites who also attend to their displays (9–11).

Many male frogs possess conspicuous vocal sacs that evolved to recycle air during the production of their advertisement calls (6). The inflation and deflation of the vocal sac additionally act as

¹Smithsonian Tropical Research Institute, Apartado 0843-03092, Balboa, Ancón, Republic of Panama. ²Institute of Biology, Leiden University, 2300 RA Leiden, Netherlands. ³Department of Integrative Biology, University of Texas, Austin, TX 78712, USA. ⁴Department of Biology, Salisbury University, Salisbury, MD 21801, USA.

*Corresponding author. E-mail: w.halfwerk@biology.leidenuniv.nl

visual cues in female attraction and male competition (12–14). Furthermore, when frogs call from the water's surface, their body movement incidentally creates surface waves or ripples which may be perceived as a third component by receivers (a tactile component in addition to the acoustic and visual components (15) (Fig. 1 and movie S1). Water ripples play a communicative role in some frog species (16, 17), but we have had no understanding of how call-induced cues are integrated with the perception of the acoustic component of the call by intended receivers and eavesdroppers.

In our study system, male túngara frogs (*Physalaemus pustulosus*) aggregate at night in shallow ponds and call to attract females (18). Males compete acoustically with other túngara males and maintain spacing by physically defending a calling site with a radius of roughly 7.5 cm against competitors (18). Males display from shallow puddles and create ripples as a by-product of call production, which can potentially be used by other males to assess the location of rivals. The frog-eating bat (*Trachops cirrhosus*) can eavesdrop on the acoustic component of the frog's call (11, 19). To reduce predation risk, frogs stop calling in response to predator cues (20), a strategy that has been shown to effectively increase localization errors by bats (21). Other bat species are able to use echolocation to detect small water droplets released into the air (splashes) or other cues produced when aquatic prey disrupt the water surface (22–24). We hypothesized that ripples produced during calling may aid frog-eating bats in the detection or localization of calling frogs, reducing the effectiveness of the frog's antipredator strategy of call cessation (20, 21).

Male túngara frogs generally show one of three behaviors in response to a nearby calling male, which is probably related to the risk of aggressive escalation and the motivation to fight (2). A challenged male can deflate his vocal sac and cease calling (but remain stationary at his call site), increase his call rate, or approach the rival to fight (18). We tested whether males used ripples associated with calling to assess rival competition. In playback experiments, males were presented with a multisensory display (sound plus ripples), sound alone, and ripples alone. We also varied the distance from the stimulus to the male [inside versus outside the physically defended range; (25) and fig. S1].

All males responded to ripples when they co-occurred with the acoustic signal (Fig. 1). On average, the call rate more than doubled during exposure to the multisensory signal as compared to the rate with sound alone [generalized linear mixed model (GLMM); $n = 22$ frogs, $df = 21$, t statistic (t) = 6.87, $P < 0.001$; Fig. 1 and movie S2]. Males did not respond to ripples alone, demonstrating that sound is the dominant signal component of the courtship behavior.

Male responses to ripples were dependent on distance. When the stimulus was within 7.5 cm of the focal male (i.e., within the zone that is physically defended), males called less in response to sound plus ripples and occasionally

ceased calling and deflated their vocal sac. When the stimulus was broadcast from 30 cm away, however, males increased their rate of response when ripples accompanied sound (GLMM, effect of distance on call cessation: $n = 18$ frogs, $df = 1$, $\chi^2 = 6.05$, $P = 0.014$; effect on call rate: $n = 18$ frogs, $df = 17$, t value = 5.79, $P < 0.001$; Fig. 1). Distance from the playback source, however, did not affect male response to only the acoustic component of the signal (call cessation: $n = 16$ frogs, $df = 1$, $\chi^2 = 0.0$, $P = 1.0$; call rate: $n = 14$ frogs, $df = 13$, t value = 1.46, $P = 0.17$).

The ripples produced during calling travel slowly in shallow water (~25 cm/s) (26) and therefore could potentially be detected by an eavesdropping predator for up to several seconds after call cessation. We tested whether bats could make use of such an aftereffect by offering bats ($n = 10$) a choice between a frog model next to a pool in which we generated the acoustic component of the call and ripples or a model that was placed next to a control pool in which the acoustic component of the call was broadcast but which lacked ripples [(25) and fig. S1]. In each trial, both stimuli were presented simultaneously and both were immediately halted when the bat flew from its perch, thereby mimicking the natural antipredator response of the frogs. Although sound immediately ceased at this point, ripple propagation continued

until the ripples reached the end of the experimental pool 2 to 3 s later.

Bats preferentially attacked the model associated with ripples (GLMM; $df = 1$, z score (z) = 2.81, $P = 0.005$; Fig. 2 and movie S3). The attack rate on the multisensory signal (sound plus ripples) increased by 36.5% as compared to the control signal (sound alone). These experiments demonstrate that bats make use of call-induced ripples, most likely through echolocation, because the experiments were conducted with no visible light and thus should have deprived the bat of visual cues.

We assessed the detection limits of echoes returning from smooth versus disturbed water surfaces by broadcasting an artificial sonar call to the experimental pools under varying angles and recording the returning echoes. Echo amplitude, and hence signal detection, strongly depends on the angle between sonar beam and water surface because of the acoustic mirror effect (24), making it unlikely that bats perceived the ripples from their perch (see also echo examples in Fig. 2). At close range, echoes returning from pools with ripples had higher variance in amplitude than echoes from control pools with smooth water surfaces (Fig. 2), generating a detectable cue for bats as they approach.

In nature, the túngara frogs' calling sites vary in the amount of clutter on or near the water surface,

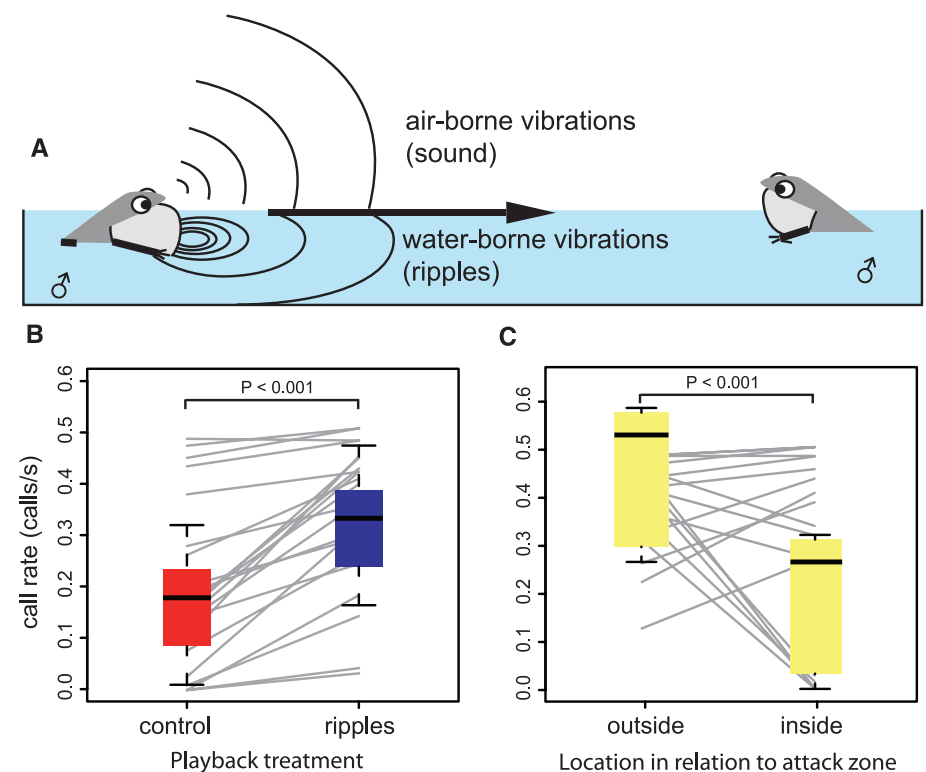


Fig. 1. Call-induced water ripples alter rival responses. (A) Calling male túngara frogs produce airborne vibrations (acoustic sound), the primary signal component, known to target males and females. Males also create waterborne vibrations (water ripples), an incidental by-product of calling from water surfaces. (B) Males increase call rates in response when ripples are added to sound playback. (C) When ripples and sound are played simultaneously from within the physically defended zone (7.5 cm), a significant proportion of males reduce their call rate, cease calling, and/or deflate, presumably to fight or flee. Shown are box plots of male call rate model estimates plus the individual responses of tested frogs (gray lines).

ranging from completely open to completely vegetated. We varied the calling environment by placing screens partially covered with leaf litter over the two experimental pools (a treatment that increases echolocation clutter) and presented bats with stimuli emanating from sites with and without clutter. Clutter treatment had a strong effect on attack preference ($df = 1$, $\chi^2 = 16.02$, $P = 0.005$, Fig. 2): When both pools were partially covered with leaf litter, the attack preference disappeared ($df = 1$, $z = -0.34$, $P > 0.97$).

Sexual selection has been responsible for generating some of the most elaborate behavioral phenotypes in the animal kingdom (1), but the evolution of such traits may be restricted by an increased risk of eavesdropping by parasites and

predators (3, 9). Understanding the specific mechanisms responsible for driving the diversification of multisensory signals is challenging. It requires demonstration of the effects of individual and combined signal components on receivers (e.g., how receivers perceptually integrate multiple signal components), and it requires an understanding of costs imposed on those signals. Our results provide three important insights into this process. First, we demonstrated that male túngara frogs attend to ripples in addition to the calls of perceived rivals. This probably improves the ability of males to make decisions about calling effort and risk relative to competition from nearby conspecifics (27). Ripples may also aid females in localization of the signaler through their integra-

tion with airborne sound (28). Second, we showed that the same signal by-product can be detected through different sensory systems by intended and unintended receivers. Detection of ripples by túngara frogs probably occurs via tactile stimulation, whereas bats detect them in the acoustic domain through echolocation. The perception of signal by-products through different senses by different receivers increases the range of eavesdroppers that can exploit the communication system. Third, predator detection of these signal by-products imposes a strong cost on signalers because of the frog's inability to halt ripple propagation (see movie S3 for a demonstration of a bat circling the ripples at some distance from the source, ~2 s after we stopped call production). This could counter the frog's main antipredator strategy of call cessation, because ripples leave a "footprint" of the frog's presence for several seconds after the male has disappeared from the acoustic channel. All signals and associated by-products produce disturbances in the environment, which can be perceived by multiple receiver species in different sensory systems, exposing the signalers to a complex array of costs and benefits.

References and Notes

1. M. Andersson, L. W. Simmons, *Trends Ecol. Evol.* **21**, 296–302 (2006).
2. J. W. Bradbury, S. L. Vehrencamp, *Principles of Animal Communication* (Sinauer, Sunderland, MA, ed. 2, 2011).
3. E. A. Hebets, D. R. Papaj, *Behav. Ecol. Sociobiol.* **57**, 197–214 (2005).
4. S. Partan, P. Marler, *Science* **283**, 1272–1273 (1999).
5. G. W. Uetz, J. A. Roberts, *Brain Behav. Evol.* **59**, 222–230 (2002).
6. R. C. Taylor, M. J. Ryan, *Science* **341**, 273–274 (2013).
7. H. McGurk, J. MacDonald, *Nature* **264**, 746–748 (1976).
8. M. E. Cummings, G. G. Rosenthal, M. J. Ryan, *Proc. R. Soc. B Biol. Sci.* **270**, 897–904 (2003).
9. M. Zuk, G. R. Kolluru, *Q. Rev. Biol.* **73**, 415–438 (1998).
10. J. A. Roberts, P. W. Taylor, G. W. Uetz, *Behav. Ecol.* **18**, 236–240 (2007).
11. K. L. Akre, H. E. Farris, A. M. Lea, R. A. Page, M. J. Ryan, *Science* **333**, 751–752 (2011).
12. P. M. Narins, D. S. Grabul, K. K. Soma, P. Gaucher, W. Hödl, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 2425–2429 (2005).
13. R. C. Taylor, B. A. Klein, J. Stein, M. J. Ryan, *Anim. Behav.* **76**, 1089–1097 (2008).
14. D. Preininger *et al.*, *Behav. Ecol. Sociobiol.* **67**, 1449–1456 (2013).
15. A. Elepfandt, A. Simm, *Biol. Behav.* **10**, 333–341 (1985).
16. G. Hobel, R. C. Kolodziej, *Behaviour* **150**, 471–483 (2013).
17. W. Walkowiak, H. Münz, *Naturwissenschaften* **72**, 49–51 (1985).
18. M. J. Ryan, *The Túngara Frog: A Study in Sexual Selection and Communication* (Univ. of Chicago Press, Chicago, 1985).
19. M. D. Tuttle, M. J. Ryan, *Science* **214**, 677–678 (1981).
20. X. E. Bernal, A. S. Rand, M. J. Ryan, *Ethology* **113**, 755–763 (2007).
21. R. A. Page, M. J. Ryan, *Anim. Behav.* **76**, 761–769 (2008).
22. K. Übernickel, M. Tschapka, E. K. V. Kalko, *Front. Physiol.* **4**, 96 (2013).
23. R. A. Suthers, *J. Exp. Zool.* **158**, 319–347 (1965).
24. B. M. Siemers, P. Stitz, H. U. Schnitzler, *J. Exp. Biol.* **204**, 3843–3854 (2001).
25. Information on materials and methods is available in the supplementary materials on Science Online.
26. H. Markl, in *Neuroethology and Behavioral Physiology: Roots and Growing Points*, H. M. Franz, Ed. (Springer-Verlag, Berlin, 1983), pp. 333–353.
27. E. R. Lewis, P. M. Narins, *Science* **227**, 187–189 (1985).
28. B. Hölldobler, J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol. **184**, 129–141 (1999).

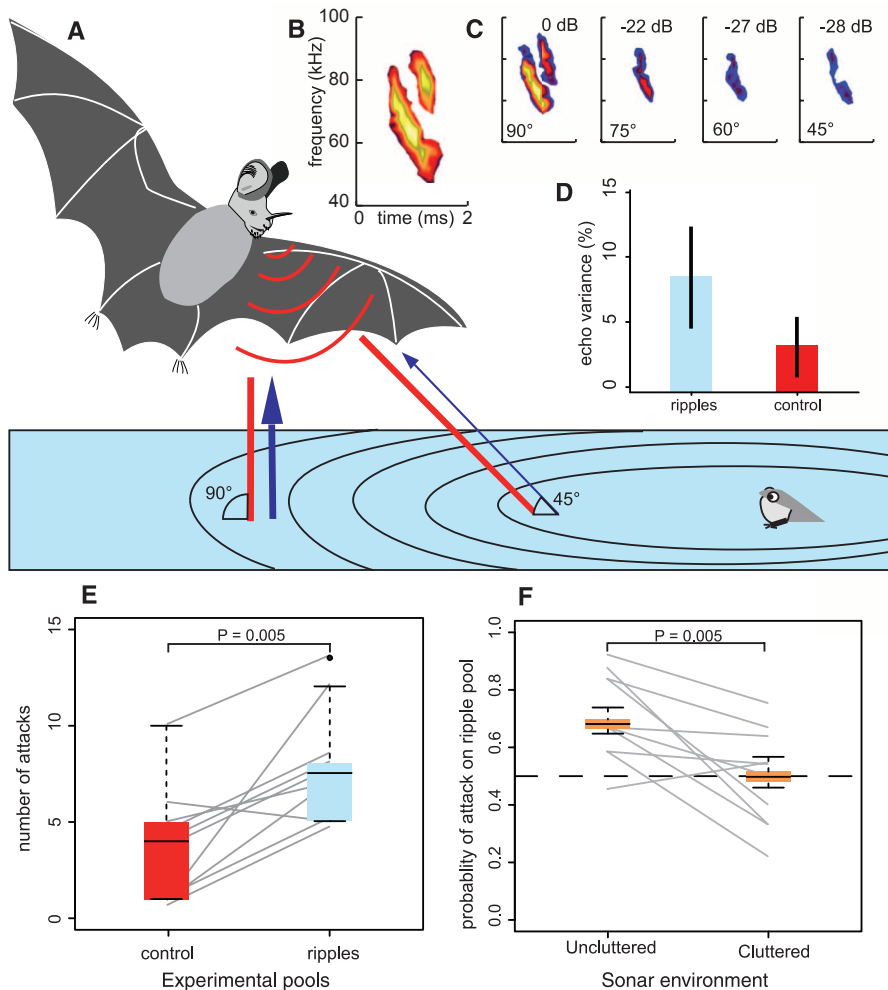


Fig. 2. Bats use call-induced ripples to hunt frogs. (A) Frog-eating bats rely partially on echolocation to hunt their prey. Water surfaces are highly reflective of echolocation signals, but the amount of returning echo (blue arrows) depends strongly on the angle between the signal propagation path (red lines) and the surface. (B) Spectrographic example of a bat's echolocation signal, with time on the x axis and frequency on the y axis. (C) Echo images derived from broadcasting a synthetic sonar call under different angles to the test pool. Echo amplitude as well as overall structure quickly decreased with decreasing angles. (D) Disturbed water surfaces during ripple playback produce higher variance in echo amplitude than do the smooth surfaces. (E) Results from a two-choice test with bats, showing a preference to attack the ripple pool over the control pool. (F) Ripple preference depends on environmental conditions. When both pools were covered with a layer of leaves (cluttered environment), preference disappeared. Graphs show mean \pm SD (D) or box plots of model estimates + individual lines [(E) and (F)].

Acknowledgments: We are grateful to M. Dixon, A. Lea, K. Ottens, J. Jacobitz, J. Finley, F. Rhebergen, and M. Shuman-Goodier for valuable help during the experiments. Comments from D. Blumstein, H. Slabbekoorn, J. Ratcliffe, and one anonymous reviewer substantially improved the manuscript, and J. Touchon and S. Dennis provided statistical advice. We thank the late Stan Rand for illuminating discussions about ripples in communication. We thank B. Klein, P. Clements, and Moeys Inc. for fabricating the pneumatic robotic frog system. The research was funded

through a Rubicon grant (825.11.026) to W.H. from the Netherlands Organization for Scientific Research; an NSF grant (IOS 1120031) to R.C.T., M.J.R., and R.A.P.; and resources made available to R.A.P. from the Smithsonian Tropical Research Institute (STRI). All research reported here complied with STRI Institutional Animal Care and Use Committee protocols. We obtained all required permits from the government of Panama. The authors report no conflict of interest. Raw data will be made available at the Dryad Data Repository (10.5061/dryad.82t1k).

Supplementary Materials

www.sciencemag.org/content/343/6169/413/suppl/DC1

Materials and Methods

Fig. S1

References (29–33)

Movies S1 to S3

16 August 2013; accepted 20 November 2013
10.1126/science.1244812

Endothelial Cell-Derived Angiopoietin-2 Controls Liver Regeneration as a Spatiotemporal Rheostat

Junhao Hu,^{1*} Kshitij Srivastava,^{1,2*} Matthias Wieland,^{1,2} Anja Runge,^{1,2} Carolin Mogler,^{1,3} Eva Besemfelder,¹ Dorothee Terhardt,¹ Marion J. Vogel,¹ Liji Cao,⁴ Claudia Korn,¹ Susanne Bartels,¹ Markus Thomas,^{1,2†} Hellmut G. Augustin^{1,2,5‡}

Liver regeneration requires spatially and temporally precisely coordinated proliferation of the two major hepatic cell populations, hepatocytes and liver sinusoidal endothelial cells (LSECs), to reconstitute liver structure and function. The underlying mechanisms of this complex molecular cross-talk remain elusive. Here, we show that the expression of Angiopoietin-2 (Ang2) in LSECs is dynamically regulated after partial hepatectomy. During the early inductive phase of liver regeneration, Ang2 down-regulation leads to reduced LSEC transforming growth factor- β 1 production, enabling hepatocyte proliferation by releasing an angiocrine proliferative brake. During the later angiogenic phase of liver regeneration, recovery of endothelial Ang2 expression enables regenerative angiogenesis by controlling LSEC vascular endothelial growth factor receptor 2 expression. The data establish LSECs as a dynamic rheostat of liver regeneration, spatiotemporally orchestrating hepatocyte and LSEC proliferation through angiocrine- and autocrine-acting Ang2, respectively.

The vascular endothelium is considered a passive cell population that acts in response to exogenous cytokines. However, recent work has shown that the endothelium can actively function as gatekeeper of tissue homeostasis. Endothelial cell-derived angiocrine signals orchestrate organogenesis during development (1, 2) and promote liver and lung regeneration in the adult (3, 4). Liver regeneration is a prototypic example of the intricate cross-talk between parenchymal cells and stromal cells (5–8). Liver sinusoidal endothelial cells (LSECs) have been shown to exert protective functions on hepatocytes (9) and promote hepatocyte proliferation during liver regeneration (3).

In order to systematically analyze the mechanisms of LSEC-regulated angiocrine growth con-

trol during liver regeneration, we isolated LSEC from sham-operated and two-thirds partial hepatectomized mice 1 day after surgery and performed transcriptomic gene expression analyses. Ninety-three genes were significantly up-regulated in LSEC upon partial hepatectomy (PHx) (table S1). Only nine genes were significantly down-regulated. Among the most strongly down-regulated LSEC genes was Angiopoietin-2 (Ang2) (Fig. 1A and table S2). Ang2 is a contextual antagonist of

the vascular receptor tyrosine kinase Tie2 and is expressed at low levels in resting endothelial cells (10, 11). Angiogenic or inflammatory endothelial activation leads to the up-regulation of Ang2 (12–14). The rapid down-regulation of LSEC Ang2 after PHx was consequently counterintuitive and prompted us to systematically study the role of LSEC-derived Ang2 during liver regeneration.

Quantitative polymerase chain reaction (PCR) analysis of Ang2 in liver lysates confirmed the rapid Ang2 down-regulation after PHx. One day after PHx, Ang2 mRNA levels were down-regulated to 18% of liver lysates from sham-operated mice. Ang2 expression thereafter steadily recovered to normal levels at day 8, when the liver restores its normal mass (Fig. 1B). The temporal pattern of Ang2 expression corresponded to the well-established pattern of hepatocyte and nonparenchymal cell proliferation after PHx (15). In mice, liver regeneration after PHx occurs rapidly by means of hepatocyte hyperplasia and hypertrophy to reach a proliferation peak as early as 48 hours after hepatectomy (16). Thereafter, hepatocyte proliferation steadily declines to baseline (15). In contrast, nonparenchymal cells, including LSECs, reach a proliferation peak 4 days after PHx, which is concomitant with the gradual recovery of Ang2. We therefore hypothesized that LSEC-derived Ang2 may negatively control hepatocyte proliferation and that Ang2 down-regulation after PHx may contribute to hepatocyte proliferation by releasing an angiocrine growth regulatory brake.

¹Division of Vascular Oncology and Metastasis, German Cancer Research Center (DKFZ), DKFZ-Center for Molecular Biology Alliance, 69120 Heidelberg, Germany. ²Department of Vascular Biology and Tumor Angiogenesis (CBTM), Medical Faculty Mannheim, Heidelberg University, 69120 Heidelberg, Germany. ³Department of Pathology, Medical Faculty Heidelberg, Heidelberg University, 69120 Heidelberg, Germany. ⁴Division of Medical Physics in Radiology, German Cancer Research Center, Heidelberg, 69120 Heidelberg, Germany. ⁵German Cancer Consortium, 69120 Heidelberg, Germany.

*These authors contributed equally to this work.

†Present address: Discovery Oncology, Pharmaceutical Research and Early Development (PRE), Roche Diagnostics GmbH, 82372 Penzberg, Germany.

‡Corresponding author. E-mail: augustin@angiogenese.de

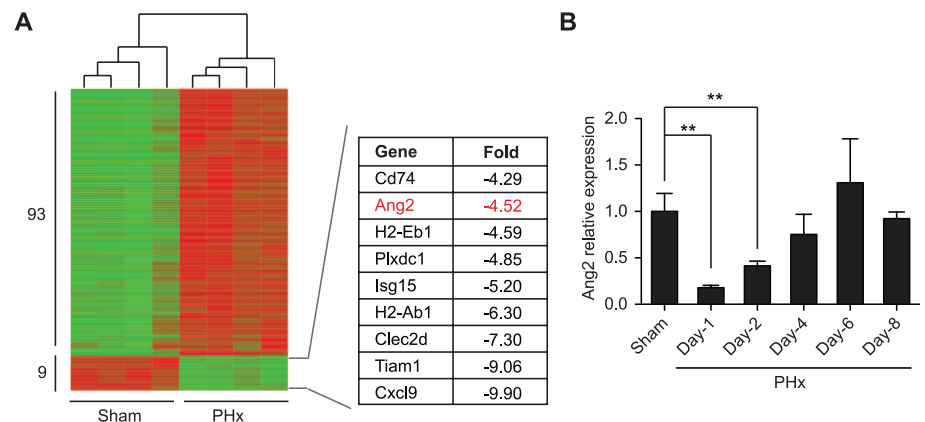


Fig. 1. Dynamics of Ang2 expression in LSECs after hepatectomy. (A) Heat map representation of significantly changed LSEC genes from sham operated and hepatectomized mice one day after surgery ($n = 4$ mice). Details of identified genes are listed in tables S1 and S2. **(B)** Temporal kinetics of Ang2 expression during liver regeneration by means of quantitative PCR analysis of mRNA from whole-liver lysate (mean \pm SD, $n = 4$ mice, $**P < 0.01$).