Anxiety-like behavior in crayfish is controlled by serotonin

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occupancy and colonization rates, which may be explained by light, humidity, and temperature conditions (Table 1 and Fig. 4F). In contrast to colonizations, extinctions were characterized by a much higher range of spatial autocorrelation (Fig. 4C), suggesting the importance of large-scale (unmeasured) environmental variation in weather, such as snow cover, for this phase.

Last, our study confirms that host resistance increases as host population density increases, with direct effects on epidemiological dynamics. In contrast to predictions from metapopulation theory (*12*), isolated host populations were more frequently infected by the pathogen than host populations in dense networks (S^H, Table 1). Moreover, pathogen colonization rate decreased and extinction rate increased as a function of host population connectivity (Table 1). This trend was particularly pronounced for the extinction probability of the pathogen in recently colonized host populations (Fig. 5A). Our experimental challenge of 22 *P. lanceolata* populations with the pathogen confirmed that resistance was significantly higher in the highly connected than in the isolated host populations (Fig. 5B). *Plantago* populations rapidly evolve resistance against the powdery mildew (*25*), and potentially higher gene flow into well-connected host populations may result in increased evolutionary potential and hence may reduce the probability of pathogen establishment and persistence. In addition to putatively higher rates of gene flow between *P. lanceolata* populations in high-density networks, an alternative, although not mutually exclusive, hypothesis is that areas supporting high-density host networks may also represent high-productivity environments for the host. In high-quality environments, hosts may be able to invest more toward disease resistance than hosts growing in areas that are nutritionally limited (*28*), resulting in resistance aggregating in high-connectivity areas of the landscape. The study of how spatial connectivity affects host-pathogen coevolution has been predominantly theoretical (*16*, *17*), but, as shown in our work, variation in disease resistance among host populations may act as a powerful barrier against disease establishment in natural systems (*24*, *29*) and may stabilize coevolutionary dynamics (*30*).

Our study provides direct evidence of spatial structure having a profound effect on the ecology and evolution of disease dynamics in natural populations. Although comparable long-term and spatially explicit ecological and evolutionary data are lacking, we suggest that metapopulation structure and spatial heterogeneity at larger geographic scales may similarly drive antagonistic and mutualistic species interactions and their coevolutionary trajectories in a range of species interactions (*31*). Many factors explaining spatiotemporal variation in disease incidence across the metapopulation also explained within host population variation in disease abundance (Table 1), suggesting that incidence data at the metapopulation scale may be sufficient for understanding disease dynamics at finer spatial scales. With the majority of epidemiological studies targeted toward understanding the growth phase of epidemics, we argue that more research should be directed toward understanding the drivers behind disease persistence at low endemic levels and the between-epidemic phase. Spatial and environmental heterogeneity, combined with the evolution of increased host resistance in high density host networks, are likely to be important components that prevent runaway dynamics of infection in nature. These insights may provide a natural blueprint for managing emerging diseases, as well as managing outbreaks that threaten sustainable agriculture and human health.

REFERENCES AND NOTES

1. D. J. Bradley, G. S. Gilbert, J. B. H. Martiny, *Ecol. Lett.* **11**, 461–469 (2008).
2. E. T. Borer, E. W. Seabloom, C. E. Mitchell, A. G. Power, *Ecol. Lett.* **13**, 810–818 (2010).
3. D. L. Smith, L. Ericson, J. J. Burdon, *J. Ecol.* **99**, 634 (2011).
4. J. Antonovics, in *Ecology, Genetics, and Evolution of Metapopulations*, I. Hanski, O. Gaggiotti, Eds. (Academic Press, San Diego, CA, 2004), pp. 471–488.
5. A.-L. Laine, I. Hanski, *J. Ecol.* **94**, 217–226 (2006).
6. D. Cressey, *Nature* **493**, 587 (2013).
7. B. A. McDonald, C. Linde, *Annu. Rev. Phytopathol.* **40**, 349–379 (2002).
8. P. K. Anderson *et al.*, *Trends Ecol. Evol.* **19**, 535–544 (2004).
9. C. Viboud *et al.*, *Science* **312**, 447–451 (2006).
10. J. O. Lloyd-Smith, S. J. Schreiber, P. E. Kopp, W. M. Getz, *Nature* **438**, 355–359 (2005).
11. A. S. Siraj *et al.*, *Science* **343**, 1154–1158 (2014).
12. I. Hanski, *Metapopulation Ecology* (Oxford Univ. Press, Oxford, 1999).
13. B. Grenfell, J. Harwood, *Trends Ecol. Evol.* **12**, 395–399 (1997).
14. P. H. Thrall *et al.*, *Ecol. Lett.* **15**, 425–435 (2012).
15. S. Gandon, Y. Michalakis, *J. Evol. Biol.* **15**, 451–462 (2002).
16. S. Gandon, S. L. Nuismer, *Am. Nat.* **173**, 212–224 (2009).
17. U. Carlsson-Granér, P. H. Thrall, *Oikos* **97**, 97–110 (2002).
18. P. H. Thrall, J. J. Burdon, *Plant Pathol.* **51**, 169–184 (2002).
19. J. D. Hoeksema, S. E. Forde, *Am. Nat.* **171**, 275–290 (2008).
20. C. Tollenaere, A.-L. Laine, *J. Evol. Biol.* **26**, 1716–1726 (2013).
21. A. J. M. Tack, A.-L. Laine, *New Phytol.* **202**, 297–308 (2014).
22. O. Ovaskainen, A.-L. Laine, *Ecology* **87**, 880–891 (2006).
23. A.-L. Laine, *J. Evol. Biol.* **20**, 1665–1673 (2007).
24. A.-L. Laine, *J. Ecol.* **92**, 990–1000 (2004).
25. A.-L. Laine, *Proc. Biol. Sci.* **273**, 267–273 (2006).
26. J. K. M. Brown, M. S. Hovmøller, *Science* **297**, 537–541 (2002).
27. D. E. Aylor, *Annu. Rev. Phytopathol.* **28**, 73–92 (1990).
28. M. A. Duffy *et al.*, *Science* **335**, 1636–1638 (2012).
29. P. H. Thrall, J. Antonovics, *Can. J. Bot.* **73**, S1249 (1995).
30. E. Decaestecker, H. De Gersem, Y. Michalakis, J. A. M. Raeymaekers, *Ecol. Lett.* **16**, 1455–1462 (2013).
31. J. N. Thompson, *The Geographic Mosaic of Coevolution* (Univ. of Chicago Press, Chicago, 2005).

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SUPPLEMENTARY MATERIALS

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COMPARATIVE BEHAVIOR

Anxiety-like behavior in crayfish is controlled by serotonin

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Anxiety, a behavioral consequence of stress, has been characterized in humans and some vertebrates, but not invertebrates. Here, we demonstrate that after exposure to stress, crayfish sustainably avoided the aversive illuminated arms of an aquatic plus-maze. This behavior was correlated with an increase in brain serotonin and was abolished by the injection of the benzodiazepine anxiolytic chlordiazepoxide. Serotonin injection into unstressed crayfish induced avoidance; again, this effect was reversed by injection with chlordiazepoxide. Our results demonstrate that crayfish exhibit a form of anxiety similar to that described in vertebrates, suggesting the conservation of several underlying mechanisms during evolution. Analyses of this ancestral behavior in a simple model reveal a new route to understanding anxiety and may alter our conceptions of the emotional status of invertebrates.

Sources of stress or danger (called stressors) provoke fear, a basic emotion, and generate immediate responses, such as escape, freezing, or aggression. Stress can also lead to anxiety, a more complex state that is considered a secondary emotion because it occurs when the stressor is absent or not clearly identified (*1*–*3*). In humans and rodents, anxiety is experienced as an anticipatory fear that facilitates coping with unexpected situations and

is revealed by a long-lasting behavioral adaptation intended to minimize threats, even in a different context and without the stressor (*1*–*3*). After the discovery of anxiolytics that act either on the serotonergic pathway or on the benzodiazepine site of γ-aminobutyric acid (GABA) receptors, anxiety has been intensively studied in humans and rodents (*4*, *5*). However, most animals are capable of perceiving danger and exhibit varying degrees of behavioral adaptation to stress (*1*). Studies on zebrafish (*6*) have extended the concept of anxiety to all vertebrates. However, although studies have described fear response after aversive conditioning in *Aplysia* or pessimistic bias after stress in bees (*7*, *8*), the characteristics of anxiety have not been fully observed in a single invertebrate model. In this study, we demonstrate that stressed crayfish (*Procambarus clarkii*) express context-independent anxiety-like behavior that can be promoted by 5HT and abolished by a benzodiazepine.

Crayfish naturally explore new environments but generally display a preference for dark places (*9*). To measure possible variations in light aversion, we designed an aquatic dark/light plus-shaped maze (D/L+maze) that was divided into two shaded ("dark") arms, two exposed ("light") arms and a starting ("middle") zone (Fig. 1, A and B, and movie S1). During a 10-min testing period, 10 variables were measured to characterize the distance walked and the number/duration of visits in the compartments (table S1). Unstressed crayfish generally explored the entire maze (Fig. 1C). Dark preference was confirmed by a higher time spent in dark zones ($P < 0.05$) (Fig. 1D), a shorter latency of the first entry ($P < 0.05$) (Fig. 1E, left), and a greater number of entries ($P < 0.001$) (Fig. 1E, right) into dark arms. Crayfish often ceased locomotion before entering a light arm and frequently retreated (movie S2), a behavior quantified by calculating the retreat ratio (RR) (table S1).

We stressed other crayfish (Fig. 2A) by exposure to repetitive electrical fields that triggered tail-flips (rapid backward movements), which were considered aversive responses (*10*, *11*). Stress induced a significant increase in blood glucose, which is a stress biomarker (fig. S1). Behavioral consequences were analyzed in the D/L+maze, where in contrast to unstressed crayfish, the stressed animals rarely explored or rapidly abandoned the light arms ($P < 0.001$, Mann-Whitney) (Fig. 2, B and C; table S2; and movie S3). In addition, the number of entries, mean duration per visit, latency to first entry into a light arm, and RR were significantly altered ($P < 0.001$, Mann-Whitney) (table S2). These changes were not due to a conditioned reflex but, in agreement with the anxiety criteria, were displayed in the absence of the stressor and in a new context.

Increasing stress duration up to 30 min decreased the time spent in the light arms, but longer exposure had no additional effect (Fig. 2D). The other measured variables also remained stable after 30 min of stress (fig. S2). Enhanced light avoidance persisted for at least 30 min and regularly returned to normal levels within 90 min (Fig. 2E and fig. S3). Therefore, the stress-induced behavioral adaptation of crayfish was sustainable, which is another criterion of anxiety.

Stress generally involves rapid mobilization of neuroendocrine centers that are considered homologous to the hypothalamo-pituitary axis of vertebrates (*12*). In crustaceans, such a center (X-organ/sinus gland) secretes hyperglycemic/steroidostatic hormones (CHH/MIH) under the control of serotonin (*13*–*15*). In crayfish, endogenous 5HT concentrations measured by means of high-performance liquid chromatography (HPLC) (*16*) were significantly elevated in the brains of stressed animals ($P < 0.01$, Mann-Whitney) (Fig. 2F) but not in their ventral cords (thoracic and abdominal ganglia), possibly because of the lower number of 5HT cells in cords than in brains (*17*, *18*). To examine the functional

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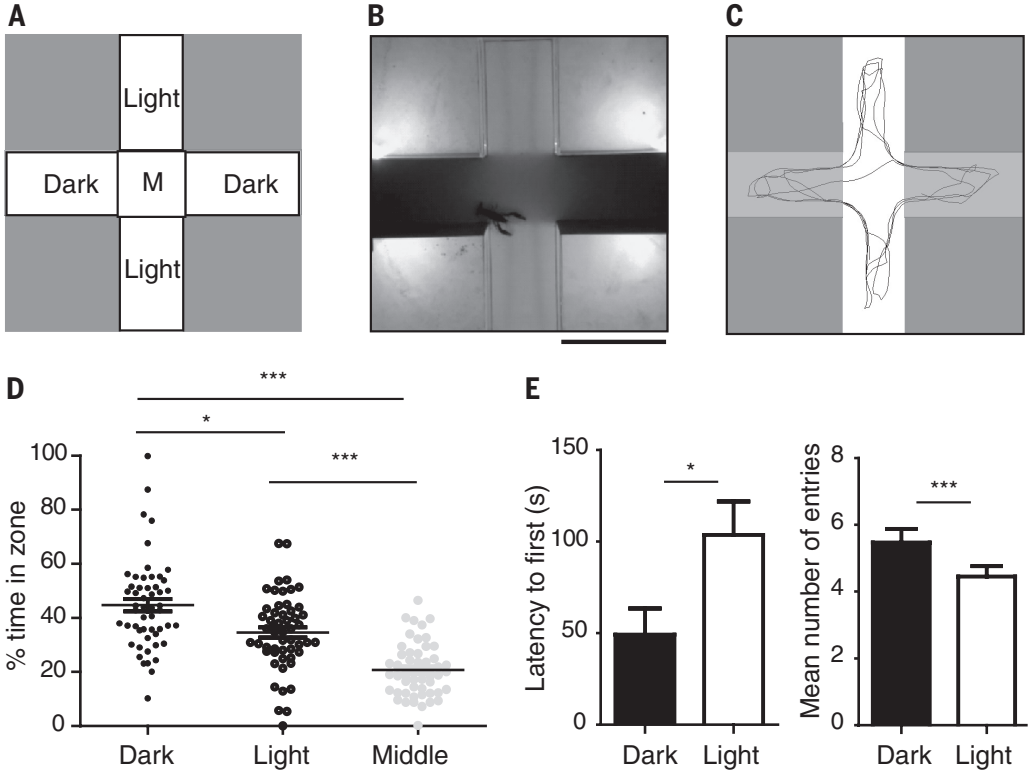


Fig. 1. Behavior of unstressed crayfish in the D/L+maze. (**A**) Illustration and (**B**) image of the D/L+maze comprising two illuminated (light) arms, two dark arms, and a middle (M) area from which the spontaneous behavior began. Scale bar, 25 cm. (**C**) An example maze route of an unstressed crayfish during the total experimental time (10 min). (**D**) Crayfish ($n = 55$) spent more time in the dark arms (43 ± 2% of total time) than in the light arms (35.7 ± 1.7%, $P < 0.05$). (**E**) (Left) The latency to the first entry into a dark arm (49 ± 14 s) was significantly shorter ($P < 0.05$) than that into a light arm (92 ± 15 s). (Right) The number of entries into dark arms (5.5 ± 0.4) was significantly greater ($P < 0.001$) than those into light arms (4.7 ± 0.3).

importance of these elevated 5HT levels, unstressed crayfish were injected with 5HT. As in other crustaceans (*15*), this bioamine was able to penetrate into the nervous structures of crayfish (supplementary materials, drug treatments) and induced a rapid increase in blood glucose (fig. S4). When transferred to the D/L+maze, in addition to reduced locomotion ($P < 0.05$) (table S2) the 5HT-injected crayfish displayed greater light avoidance as compared with that of saline-injected unstressed animals ($P < 0.05$, regarding percent of time in light arms, mean number of entries, latency to first entry in a light arm, and RR) (Fig. 2, G and H), similar to electrically stressed animals ($P > 0.05$ for all variables; compare with Fig. 2, B and C). In contrast, a mixture of mianserin and methysergide, two broad-spectrum serotonin antagonists (*14*, *19*, *20*), prevented avoidance behavior when administered before electric field exposure [$P > 0.05$, compare with unstressed+saline for all variables (table S2)].

We next examined whether a well-characterized anxiolytic drug could abolish the behavioral changes induced by electric fields or 5HT injections. Chlordiazepoxide (CDZ), a potent benzodiazepine (*5*), prevented changes in exploratory behavior in animals previously submitted to either electric fields (Fig. 3A, compare with Fig. 2B; and movie S4) or 5HT injection (Fig. 3B, compare with Fig. 2G) and restored the time spent in the light arms ($P < 0.05$, Dunn's test, compared with stressed+saline or unstressed+5HT) (Fig. 3, C and D), the latency to the first entry into a light arm, and RR [$P < 0.05$, Dunn's test, compared with stressed+saline or unstressed+5HT (table S2)]. This effect of CDZ was dose-dependent (fig. S5) and specific to avoidance behavior because it did not prevent the increase in glucose levels (fig. S6). When administered to unstressed crayfish, CDZ had no significant effect on time spent in light arms or on locomotion performance (table S2).

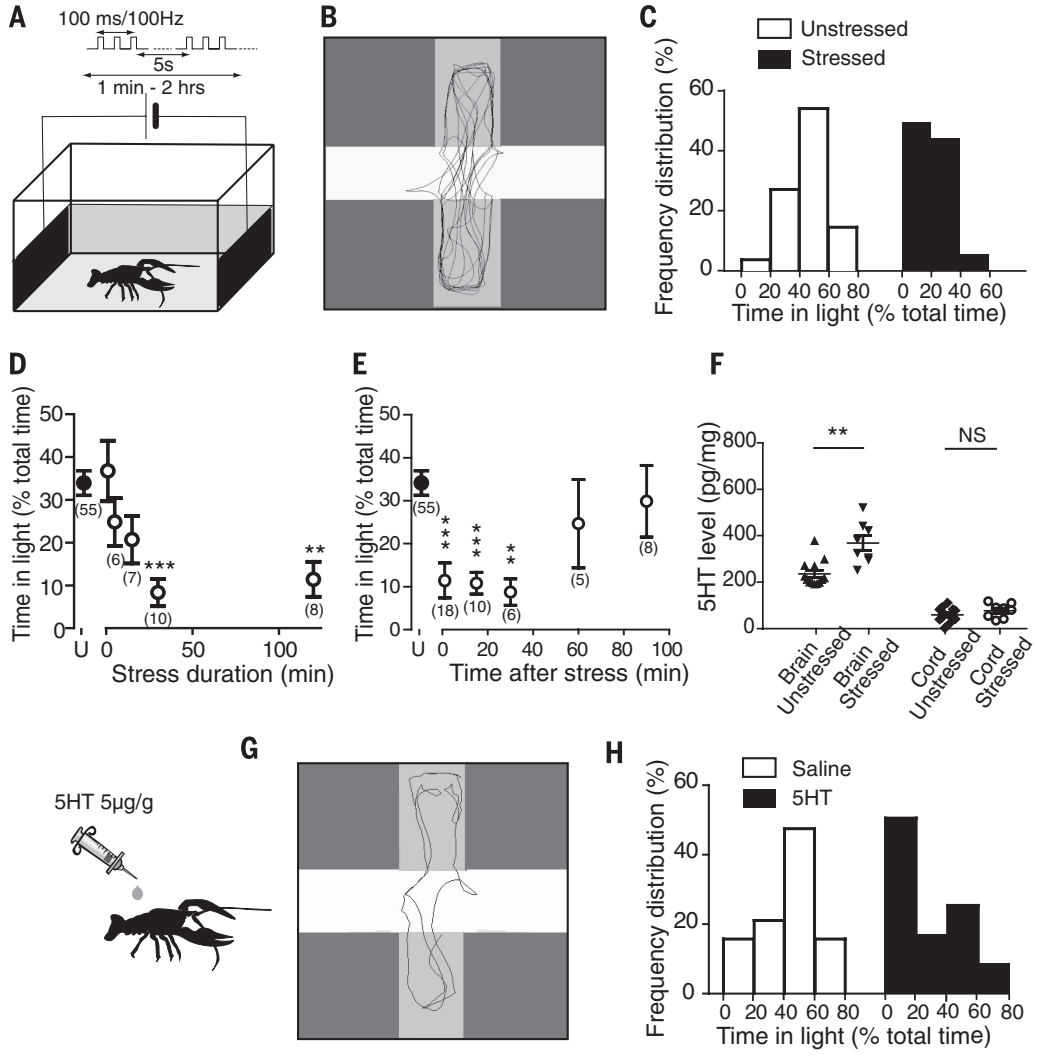
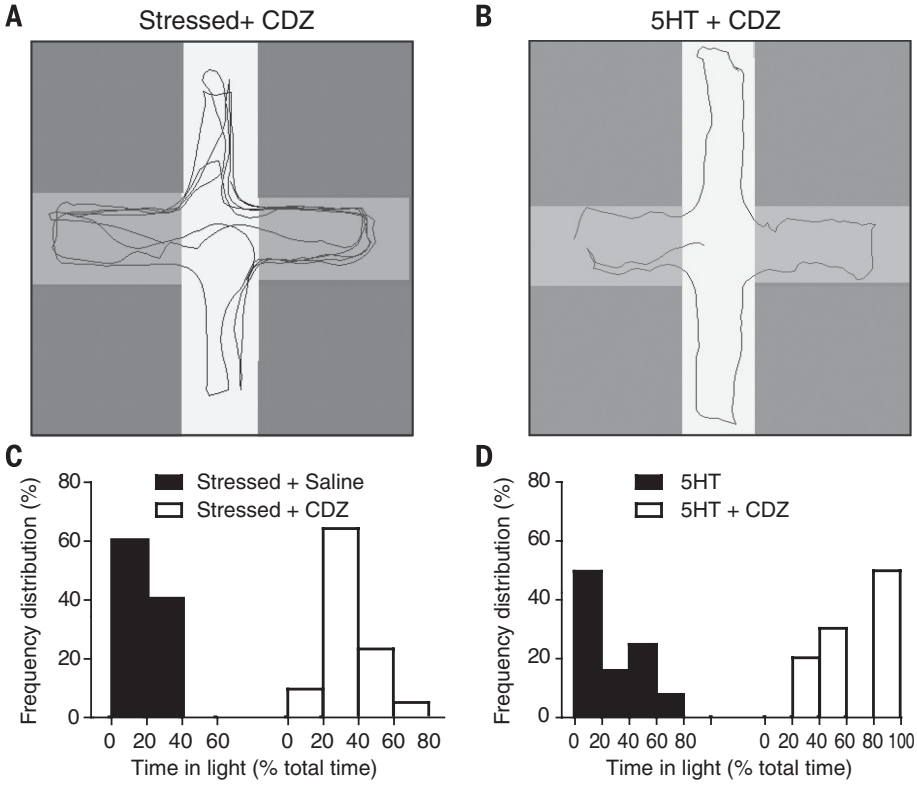


Fig. 2. Crayfish develop 5HT-dependent light avoidance after exposure to stress. (**A**) Experimental procedure for stress induction in crayfish (supplementary materials). (**B**) An example crayfish route after a 30-min exposure to an electric field. Walking occurred nearly exclusively in the dark arms. (**C**) Frequency distribution histograms of the percent time spent in light arms by stressed and unstressed crayfish. (**D**) Effect of stress duration on time spent in light arms (U = unstressed; P versus unstressed < 0.001 after 30 min and P versus unstressed < 0.01, after 2 hours of stress, Dunn's test). (**E**) Time course of behavioral changes (as measured by time spent in light arms) after exposure to a 30-min stressful experience. Crayfish recovered "normal" behavior after 90 min (U = unstressed; P versus unstressed > 0.05, Dunn's test). The number of animals (n) is in parentheses in (D) and (E). (**F**) Serotonin concentrations (in picograms per milligrams of fresh weight) measured by means of HPLC in the brain and ventral cord of unstressed and stressed crayfish. Brain concentrations of 5HT were significantly higher in stressed than in unstressed animals. (**G**) After injection of 5 µg/g 5HT into the hemolymph, the crayfish route was similar to that of stressed crayfish. (**H**) The frequency distribution histograms of percent time in light arms for saline- and 5HT-injected crayfish were similar to those for (C) unstressed and stressed crayfish, respectively.

Fig. 3. Injection of benzodiazepine suppresses stress- and 5HT-induced light avoidance. (**A** and **B**) Typical crayfish routes either (A) after 30 min of stress followed by CDZ injection or (B) after 5HT and CDZ injection. In these examples, the crayfish entered all arms, similar to unstressed animals. (**C**) Frequency distribution histograms of percent time in the light arms for stressed crayfish injected with either saline (black bars) or CDZ (white bars). (**D**) Frequency distribution histograms of percent time in the light arms for the 5HT-injected crayfish (black bars) and the crayfish injected with 5HT and CDZ (white bars).



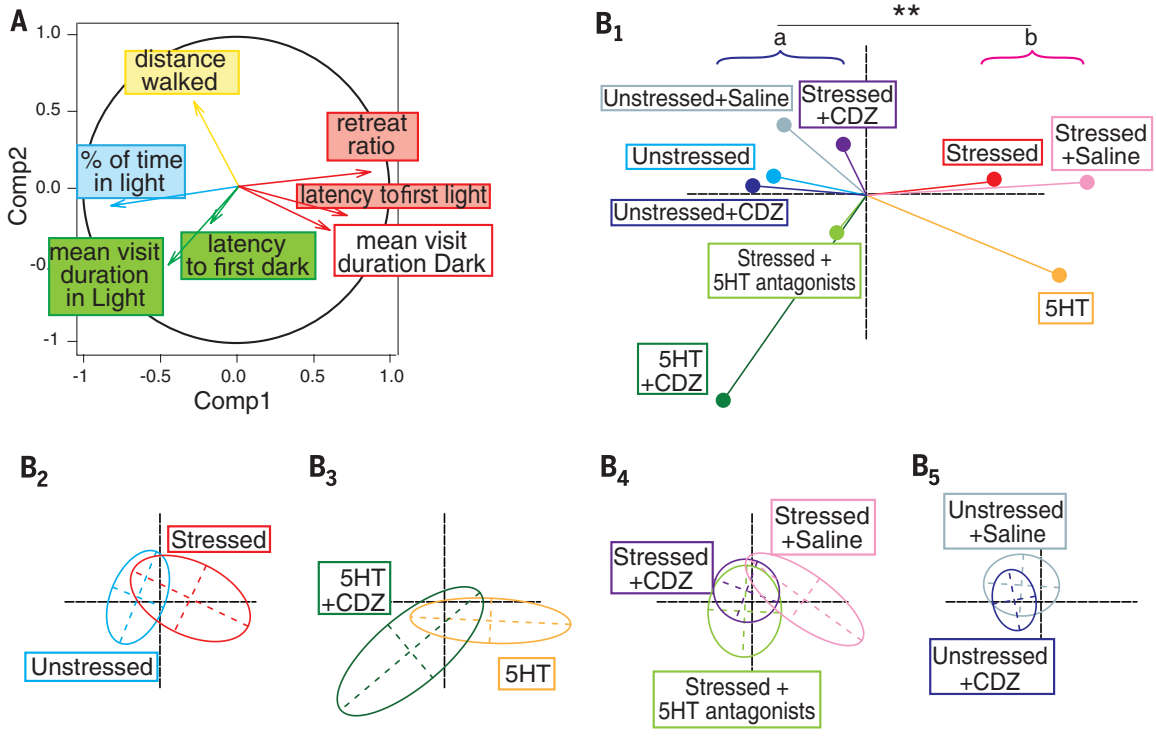


Fig. 4. PCA of the seven behavioral variables measured in experimental groups. (**A**) Contribution of the uncorrelated variables to the variances of the first and second components. (**B1**) Projection of group barycenters into the plane of the first and second components. (**B2** to **B5**) The envelopes of selected groups from the same PCA are presented separately in order to facilitate comparisons. (B2) Stressed versus unstressed crayfish. (B3) Crayfish injected with 5HT alone or in combination with CDZ. (B4) Stressed crayfish injected with saline, CDZ, or 5HT antagonists. (B5) Unstressed crayfish injected with saline or CDZ.

To compare all experimental groups, we performed principal component analysis (PCA) based on the seven uncorrelated variables (table S1, asterisk). The PCA enabled the representation of the variables (Fig. 4A) and experimental groups (Fig. 4B) on two orthogonal axes (supplementary materials, PCA). The first component (Fig. 4, x axis) can be interpreted as the anxiety level. Three groups (the stressed, stressed injected with saline, and 5HT-injected unstressed animals) exhibited substantially greater levels of anxiety (Fig. 4B1, positive x values of barycenters) and were significantly different from the other groups (Fig. 4B1, a/b groups, and table S2, a/b groups in PCA column). The stressed groups injected with either CDZ or 5HT antagonists were not significantly different from the unstressed group (and the other "a" groups). The groups injected with either 5HT or 5HT and CDZ had negative y values and were therefore separated from the other groups in the second component, mainly because of the inhibitory effects of 5HT on crustacean locomotion (*21*). Thus, 5HT had a CDZ-sensitive anxiogenic effect that was distinct from its inhibitory (and CDZ-independent) effect on locomotion.

The first conclusion of this study is that the stress-induced avoidance behavior in crayfish exhibits striking homologies with vertebrate anxiety. In the elevated plus-maze, a paradigm similar to the D/L+maze, the behavior of rodents after exposure to electric shocks (*22*) resembles that of crayfish—a context-independent and durable avoidance behavior. Similar to the risk assessment behavior described in rodents (*23*), the stressed crayfish also stopped before entering the aversive arms and frequently decided not to enter. Furthermore, as in rodents, crayfish anxiety-like behavior was sensitive to systemically applied CDZ, a potent benzodiazepine anxiolytic that modulates vertebrate GABA type A receptors (*5*). In crustaceans as in other animals, GABA is a fundamental inhibitory neurotransmitter (*24*) whose receptors are also sensitive to benzodiazepines (*25*). Our results suggest that GABA is involved in the regulation of crayfish anxiety-like behavior.

The second conclusion of this study concerns the role played by 5HT. Serotonin, already involved in the control of stress response and arousal in crustaceans, triggers the rapid secretion of CHH and the mobilization of carbohydrate reserves (*14*, *15*). Moreover, 5HT is frequently involved in the control of aggression (*26*), in which CHH also participates (*27*), implying a link between stress response and aggression. Beside the metabolic aspect of stress response, the present study shows that 5HT can also induce anxiety-like behavior in crayfish, interacting with GABA signaling (fig. S7, schematic diagram). In vertebrates, the relationships between 5HT and anxiety appear to be more complex; a classic hypothesis considering that 5HT-promoted anxiety has been contradicted by many studies (*4*). In this context, the crayfish represents a new model that might provide insights into the mechanisms underlying anxiety that have been conserved during evolution. Our results also emphasize the ability of an invertebrate to exhibit a state that is similar to a mammalian emotion but which likely arose early during the evolution of metazoans.

REFERENCES AND NOTES

1. C. Belzung, P. Philippot, *Neural Plast.* **2007**, 59676 (2007).
2. D. C. Blanchard, R. J. Blanchard, in *Handbook of Anxiety and Fear*, R. J. Blanchard, D. C. Blanchard, G. Griebel, D. Nutt, Eds. (Academic Press, New York, 2008).
3. T. Steimer, *Dialogues Clin. Neurosci.* **13**, 495–506 (2011).
4. S. L. Handley, J. W. McBlane, *Psychopharmacol. (Berl.)* **112**, 13–20 (1993).
5. F. López-Muñoz, C. Alamo, P. García-García, *J. Anxiety Disord.* **25**, 554–562 (2011).
6. R. J. Egan *et al.*, *Behav. Brain Res.* **205**, 38–44 (2009).
7. E. T. Walters, T. J. Carew, E. R. Kandel, *Science* **211**, 504–506 (1981).
8. M. Bateson, S. Desire, S. E. Gartside, G. A. Wright, *Curr. Biol.* **21**, 1070–1073 (2011).
9. S. Yamane, M. Takahata, *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.* **188**, 13–23 (2002).
10. D. H. Edwards, W. J. Heitler, F. B. Krasne, *Trends Neurosci.* **22**, 153–161 (1999).
11. N. Kawai, R. Kono, S. Sugimoto, *Behav. Brain Res.* **150**, 229–237 (2004).
12. V. Hartenstein, *J. Endocrinol.* **190**, 555–570 (2006).
13. M. P. Mattson, E. Spaziani, *Gen. Comp. Endocrinol.* **62**, 419–427 (1986).
14. C. Y. Lee, P. F. Yang, H. S. Zou, *Physiol. Biochem. Zool.* **74**, 376–382 (2001).
15. S. Lorenzon, P. Edomi, P. G. Giulianini, R. Mettulio, E. A. Ferrero, *J. Exp. Biol.* **208**, 3341–3347 (2005).
16. P. De Deurwaerdère, L. Stinus, U. Spampinato, *J. Neurosci.* **18**, 6528–6538 (1998).
17. K. Langworthy, S. Helluy, J. Benton, B. Beltz, *Cell Tissue Res.* **288**, 191–206 (1997).
18. L. Rodriguez-Sosa, A. Picones, G. Rosete, S. Arechigay, *J. Exp. Biol.* **200**, 3067–3077 (1997).
19. L. Rodriguez-Sosa *et al.*, *Comp. Biochem. Physiol. C* **142**, 220–230 (2006).
20. Y. Momohara, A. Kanai, T. Nagayama, *PLOS ONE* **8**, e74489 (2013).
21. H. V. Peeke, G. S. Blank, M. H. Figler, E. S. Chang, *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.* **186**, 575–582 (2000).
22. S. Pellow, P. Chopin, S. E. File, M. Briley, *J. Neurosci. Methods* **14**, 149–167 (1985).
23. R. J. Rodgers, A. Dalvi, *Neurosci. Biobehav. Rev.* **21**, 801–810 (1997).
24. E. Florey, *Can. J. Physiol. Pharmacol.* **69**, 1049–1056 (1991).
25. M. J. Snyder, S. Watson, H. V. S. Peeke, *Mar. Freshwat. Behav. Physiol.* **34**, 37–51 (2000).
26. R. Huber, K. Smith, A. Delago, K. Isaksson, E. A. Kravitz, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 5939–5942 (1997).
27. L. Aquiloni *et al.*, *PLOS ONE* **7**, e50047 (2012).

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SUPPLEMENTARY MATERIALS

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Movies S1 to S4

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