immorality evokes this emotion in addition to others such as anger: Whereas anger is associated with approach motivation (*29*), disgust may motivate vigorous withdrawal (*8*). Thus, unfair offers may be received like a plate of spoiled food. This turning away or rejection of unfair actions may also extend to later avoidance of transgressors.

The ability to detect and avoid toxins appears to be very ancient: Sea anemones, which evolved about 500 million years ago, evert their gastrovascular cavities in response to being fed a bitter substance (*30*). That a system with the ancient and critical adaptive function of rejecting toxic foods should be brought to bear in the moral sphere speaks to the vital importance of regulating social behavior for human beings. Although the stimulus triggers for this rejection mechanism may have shifted far from their chemical sensory origins to the moral domain, the basic behavioral program of oral rejection appears to have been conserved. Thus, the metaphorical "bad taste" left by moral transgressions may genuinely have its origins in oral distaste.

References and Notes

1. E. Turiel, M. Killen, C. C. Helwig, in *The Emergence of Morality in Young Children*, J. Kagan, S. Lamb, Eds. (Univ. of Chicago Press, Chicago, 1987), pp. 155–243.
2. L. Kohlberg, in *Handbook of Socialization Theory and Research*, D. A. Goslin, Ed. (Rand McNally, Chicago, 1969), pp. 347–380.
3. J. D. Greene, R. B. Sommerville, L. E. Nystrom, J. M. Darley, J. D. Cohen, *Science* **293**, 2105 (2001).
4. M. Koenigs *et al.*, *Nature* **446**, 908 (2007).
5. S. Schnall, J. Haidt, G. L. Clore, A. H. Jordan, *Pers. Soc. Psychol. Bull.* **34**, 1096 (2008).
6. J. Haidt, *Science* **316**, 998 (2007).
7. J. Greene, J. Haidt, *Trends Cogn. Sci.* **6**, 517 (2002).
8. P. Rozin, J. Haidt, C. McCauley, in *Handbook of Emotions*, M. Lewis, J. M. Haviland-Jones, Eds. (Guilford, New York, 2000), pp. 637–653.
9. P. Rozin, L. Lowery, S. Imada, J. Haidt, *J. Pers. Soc. Psychol.* **76**, 574 (1999).
10. A. G. Sanfey, J. K. Rilling, J. A. Aronson, L. E. Nystrom, J. D. Cohen, *Science* **300**, 1755 (2003).
11. P. Bloom, *Descartes' Baby: How the Science of Child Development Explains What Makes Us Human* (Basic Books, New York, 2004).
12. D. Jones, *Nature* **447**, 768 (2007).
13. P. Rozin, L. Lowery, R. Ebert, *J. Pers. Soc. Psychol.* **66**, 870 (1994).
14. R. L. Nabi, *Cogn. Emotion* **16**, 695 (2002).
15. J. Moll *et al.*, *Cogn. Behav. Neurol.* **18**, 68 (2005).
16. M. L. Phillips *et al.*, *Nature* **389**, 495 (1997).
17. A. J. Calder, J. Keane, F. Manes, N. Antoun, A. W. Young, *Nat. Neurosci.* **3**, 1077 (2000).
18. A. R. Damasio *et al.*, *Nat. Neurosci.* **3**, 1049 (2000).
19. H. D. Critchley, S. Wiens, P. Rotshtein, A. Ohman, R. J. Dolan, *Nat. Neurosci.* **7**, 189 (2004).
20. A. Simmons, S. C. Matthews, M. P. Paulus, M. B. Stein, *Neurosci. Lett.* **430**, 92 (2008).
21. C. Darwin, *The Expression of the Emotions in Man and Animals* (HarperCollins, London, 1872/1998).
22. J. M. Susskind *et al.*, *Nat. Neurosci.* **11**, 843 (2008).
23. L. G. Tassinary, J. T. Cacioppo, in *Handbook of Psychophysiology*, J. T. Cacioppo, L. G. Tassinary, G. G. Berntson, Eds. (Cambridge Univ. Press, Cambridge, 2000), pp. 163–198.
24. See supporting material on *Science* Online.
25. P. Ekman, W. Friesen, J. C. Hager, *Facial Action Coding System* (Research Nexus, Salt Lake City, 2002).
26. S. R. Vrana, *Psychophysiology* **30**, 279 (1993).
27. J. P. Henrich, *Foundations of Human Sociality: Economic Experiments and Ethnographic Evidence from Fifteen Small-Scale Societies* (Oxford Univ. Press, Oxford, 2004).
28. D. Matsumoto, P. Ekman, *Japanese and Caucasian Facial Expressions of Emotion (JACFEE)* [Slides] (Intercultural and Emotion Research Laboratory, Department of Psychology, San Francisco State University, 1988).
29. E. Harmon-Jones, *Pers. Individ. Differ.* **35**, 995 (2003).
30. J. Garcia, W. G. Hankins, in *Olfaction and Taste V*, D. A. Denton, J. P. Coghlan, Eds. (Academic Press, New York, 1975), pp. 39–45.
31. G. R. Loftus, M. E. Masson, *Psychon. Bull. Rev.* **1**, 476 (1994).

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Blue or Red? Exploring the Effect of Color on Cognitive Task Performances

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Existing research reports inconsistent findings with regard to the effect of color on cognitive task performances. Some research suggests that blue or green leads to better performances than red; other studies record the opposite. Current work reconciles this discrepancy. We demonstrate that red (versus blue) color induces primarily an avoidance (versus approach) motivation (study 1, $n = 69$) and that red enhances performance on a detail-oriented task, whereas blue enhances performance on a creative task (studies 2 and 3, $n = 208$ and 118). Further, we replicate these results in the domains of product design (study 4, $n = 42$) and persuasive message evaluation (study 5, $n = 161$) and show that these effects occur outside of individuals' consciousness (study 6, $n = 68$). We also provide process evidence suggesting that the activation of alternative motivations mediates the effect of color on cognitive task performances.

Color is a fundamental aspect of human perception, and its effects on cognition and behavior have intrigued generations of researchers. Although a large amount of research has been done in this domain, the psychological processes through which color operates have not been explored fully. As a result, the field has observed certain conflicting results. One inconsistency, which is the focus of this report, concerns the effect of color on cognitive task performance. Most research examining this topic has focused on two of the three primary colors—red versus blue (or green). Some have proposed that red enhances cognitive task performance as compared with blue or green (*1*, *2*); others have shown exactly the opposite (*3*, *4*).

This report details our effort to understand the theory behind the psychological process through which color affects cognitive task performances. Based on our theorizing, we are able to reconcile the above-described inconsistency. We demonstrate that red and blue activate different motivations and consequently enhance performances on different types of cognitive tasks. In line with most of the extant research, we limit our research to the two primary colors, red and blue.

Color theorists believe that color influences cognition and behavior through learned associations (*3*). When people repeatedly encounter situations where different colors are accompanied by particular experiences and/or concepts, they form specific associations to colors. Red and blue have been shown to have different associations within the cognitive domain. Red is often associated with dangers and mistakes [e.g., errors that are circled with a red ink pen, stop signs, and warnings (*3*)]. Claims have been made linking the color red to the highest level of hazard and also the highest level of compliance (*5*, *6*). In contrast, blue is often associated with openness, peace, and tranquility [e.g., ocean and sky (*7*)]. A word association test confirmed that people indeed generate these different associations to red versus blue color in the cognitive task domain (*8*, *9*).

We propose that these different associations related to red versus blue color can induce alternative motivations. Specifically, red, because of its association with dangers and mistakes, should activate an avoidance motivation, which has been shown to make people more vigilant and risk-averse (*10*–*12*). Thus, red, compared with blue, should enhance performance on detail-oriented tasks (i.e., tasks that require focused, careful attention). In contrast, because blue is usually associated with openness, peace, and tranquility, it is likely to activate an approach motivation, because these associations signal a benign environment that encourages people to use innovative as opposed to "tried-and-true" problem-solving strategies (*13*). Indeed, an approach motivation has been shown to make people behave in a more

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explorative, risky manner (*10*, *11*). Thus, blue versus red should enhance performance on creative tasks.

We report six studies (*14*) that offered systematic support to our hypotheses. Most studies were conducted on computers, and color was manipulated through the background screen color. Color is usually defined along three dimensions: hue (the pigment of the color, e.g., blue, red, etc.), chroma (saturation of color), and value (degree of darkness or lightness of the color) (*15*). In order to reduce confounds and to be consistent with prior research, we manipulated only hue (i.e., red versus blue) and kept chroma and value constant. For computer-based studies, we employed the HSL (hue-saturation-lightness) scheme (red: hue = 0, saturation = 240, lightness = 120; blue: hue = 160, saturation = 240, lightness = 120) (*8*) (table S1). To provide a baseline for comparison, we also included a neutral condition in some studies where computer background color was set to be white.

Study 1 tested our hypothesis that red color will induce primarily an avoidance motivation, whereas blue will activate an approach motivation. Sixty-nine participants were randomly assigned to the red, blue, or neutral background color condition and completed a computer-based study that consisted of two tasks. In the first task, participants solved a series of 12 anagrams, with three of them having target words related to avoidance motivation (e.g., prevent), another three having target words related to approach motivation (e.g., adventure) (*16*, *17*), and the remaining six that were unrelated to either motivation (e.g., computer) (*8*). The response times for each type of correctly solved anagrams were averaged to create three reaction time (RT) indices—avoidance, approach, and neutral RT indices. Faster reaction time to approach-related (or avoidance-related) anagrams would imply a stronger activation of an approach (or avoidance) motivation (*18*). As anticipated (Fig. 1), for approach-related anagrams, those in the blue condition [10.93 ± 5.51 s (mean ± SD)] responded faster than those in the red [18.53 ± 12.25 s; $t(66) = 2.81$, $P < 0.01$; Cohen's $d = 0.81$] or neutral condition [17.50 ± 9.17 s; $t(66) = -2.29$, $P < 0.03$; Cohen's $d = 0.91$]. For avoidance-related anagrams, the reverse pattern appeared, such that those in the red condition (10.40 ± 3.64 s) responded faster than those in the blue [20.39 ± 14.73 s; $t(66) = -3.21$, $P < 0.01$; Cohen's $d = 0.96$] or neutral condition [19.14 ± 11.48 s; $t(66) = -2.67$, $P < 0.01$; Cohen's $d = 1.1$]. For neutral anagrams, however, no effect of color was observed (red, 10.56 ± 5.48 s; blue, 12.64 ± 7.53 s; neutral, 11.58 ± 4.37 s; $t < 1$).

In the second task, participants read descriptions of three pairs of brands and reported their preferences along a scale from 1 (prefer brand A) to 7 (prefer brand B). Within each pair, one brand highlighted a negative outcome people try to avoid, whereas the other brand highlighted a positive outcome people try to approach. For example, one pair featured two brands of toothpastes, with brand A being particularly good for cavity prevention (avoidance focused), and brand B being particularly good for tooth whitening (approach focused) (*8*). Across three pairs, we found that those in the blue color condition (4.03 ± 1.55) indicated greater preference for brands that were approach-oriented (highlighting positive benefits) than those in the red [2.79 ± 1.65; $t(66) = -2.80$, $P < 0.01$; Cohen's $d = 0.79$] and the neutral condition [3.05 ± 1.43; $t(66) = 2.08$, $P < 0.05$; Cohen's $d = 0.67$]. Thus, this study demonstrated that within a cognitive task domain, red (versus blue) can activate an avoidance (versus approach) motivation. A post hoc study ruled out mood as an alternative explanation (*8*).

The next two studies tested whether red (versus blue), because of its activation of avoidance (versus approach) motivation, enhances performance on a detail-oriented (versus a creative) task. Study 2 ($n = 208$) contained two tasks, a detail-oriented and a creative task. A set of participants completed the detailed-oriented task (i.e., a memory exercise) presented on computers with red, blue, or neutral background color. They studied a list of 36 words for 2 min and were asked to recall as many words as they could after a 20-min delay. Three measures confirmed that red indeed enhanced performance on this memory task. Those in the red condition (15.89 ± 5.90) recalled more correct items than those in the blue condition [12.31 ± 5.48; $t(100) = 2.50$, $P < 0.02$; Cohen's $d = 0.64$] (Fig. 2). Furthermore, blue led to more false recalls (0.86 ± 1.29) than red [0.34 ± 0.64; $t(100) = -2.42$, $P < 0.02$; Cohen's $d = 0.52$] or neutral [0.38 ± 0.55; $t(100) = 2.21$, $P < 0.03$; Cohen's $d = 0.48$] condition. These two measures have been shown to reflect people's attention to details (*19*, *20*). However, color manipulation did not affect the total number of items recalled ($P > 0.11$) (*8*).

Another set of participants completed a creative task where they were asked to generate as many creative uses for a brick as they could think of within 1 min (*21*). Consistent with prior research (*21*), each participant's responses were coded into three categories: (i) total number of uses generated, (ii) mean creativity score as rated by a panel of judges, and (iii) total number of creative uses. Participants in the three color conditions produced equal number of uses in total ($F < 1$; red, 4.83 ± 2.31; blue, 4.67 ± 2.62; neutral, 4.94 ± 1.68). However, the quality of these uses differed by color conditions. Those in the blue condition (3.97 ± 0.99) demonstrated a higher mean creativity score than those in the red [3.39 ± 0.97; $t(102) = -2.81$, $P < 0.01$; Cohen's $d = 0.6$] or neutral color condition [3.50 ± 0.63; $t(102) = 2.23$, $P < 0.03$; Cohen's $d = 0.57$] (Fig. 3). Similarly, those in the blue (1.64 ± 1.46) condition produced more creative uses than those in the red [0.86 ± 0.97; $t(102) = -2.93$, $P < 0.01$; Cohen's $d = 0.64$] or neutral condition [0.91 ± 0.83; $t(102) = 2.70$, $P < 0.01$; Cohen's $d = 0.62$] (*8*). Findings from this study suggest that, although color did not affect the amount of processing, as shown in the equal recall level for the memory task and comparable uses generated for the brick task, it affected the quality of responses, i.e., red led to superior performances on detail-oriented tasks and blue, on creative tasks.

Study 3 ($n = 118$) tested the generalizability of results observed in study 2 by using two different tasks. Moreover, it aimed to demonstrate that the activation of avoidance or approach motivations is the underlying force that drives our results. As in study 2, this study was computer-based, and color was manipulated using the computer background screen color. The detail-oriented task in this study was a proofreading task (*22*). Participants examined five sets of items, with each set containing a pair of names or addresses, which were either identical or slightly different (*8*). Participants' task was to judge whether items within each pair were identical or not. To assess whether color-induced motivations drive our expected effects, we also asked participants to answer three questions concerning the extent to which they focused on accuracy (mistake-avoidance motivation) versus speed (approach motivation). Results revealed that red color condition (4.33 ± 0.77) led to more correct responses than blue [3.53 ± 0.80;

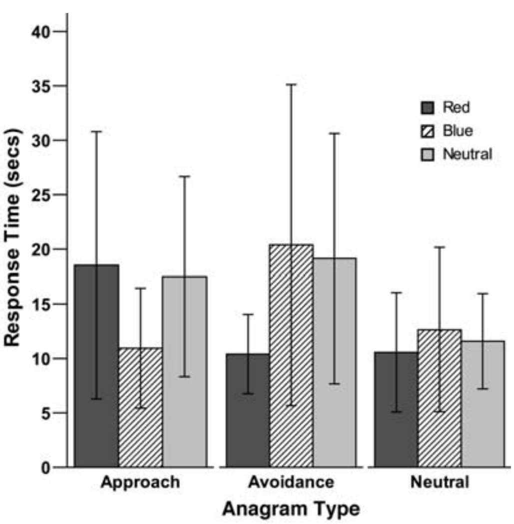

Fig. 1. Participants' response times to approach-related, avoidance-related, and neutral anagrams under red, blue, and neutral color conditions (study 1). $F_{2,66} = 8.79$, $P < 0.001$. Error bars, ±1.00 SD.

$t(51) = 2.49$, $P < 0.02$; Cohen's $d = 1.05$] or neutral [3.68 ± 1.20; $t(51) = 2.07$, $P < 0.05$; Cohen's $d = 0.66$] condition ($F_{2,51} = 3.56$, $P < 0.04$). Further, mediation analysis (*23*) revealed that approach versus avoidance motivations were indeed the driving force for the observed effect.

The Remote Associates Test (RAT), which is widely used as a test of creative thinking (*24*), was used as the creative task. Each RAT item consists of three or four stimulus words (e.g., "Shelf," "Read," and "End") that are in some way related to a fourth or fifth unreported word (e.g., "Book"). Participants were presented with five RAT items and were asked to determine what the target words were. As predicted, those in the blue condition (4.00 ± 0.74) produced more correct answers (thus exhibiting higher creativity) than those in the red [3.45 ± 0.89; $t(61) = -2.35$, $P < 0.03$; Cohen's $d = 0.69$] or neutral [3.38 ± 0.67; $t(61) = 2.67$, $P < 0.01$; Cohen's $d = 0.9$] condition ($F_{2,61} = 4.33$, $P < 0.02$). Mediation analysis again confirmed that the alternative motivations activated by color drive the observed effect (*8*).

Study 4 aimed to further extend the previous studies by using a single task that could examine both people's creativity level and their attention to details. For this purpose, participants were presented with a sheet of paper with drawings of 20 different parts (fig. S1). Participants ($n = 42$) were required to use any five parts and draw a design of a toy a child (age 5 to 11) could use to play with (*8*, *25*). Unlike previous studies, the color manipulation in this study was done by presenting the 20 parts either in red or blue color. Twelve judges evaluated black-and-white copies of each design on two dimensions, one assessing the originality and novelty (reflecting creativity level) and the other assessing the practicality and appropriateness (reflecting attention to details) of the design. The correlation between these two dimensions was 0.29 ($P = 0.08$) and interjudge reliability was 0.75 for originality and 0.83 for appropriateness. As expected, toys designed by those in the red color condition were judged to be more practical and appropriate (3.47 ± 0.79) than those in the blue condition (2.95 ± 0.88; $F_{1,40} = 4.16$, $P < 0.05$; Cohen's $d = 0.64$), but were judged less original and novel (2.94 ± 0.55) than those in the blue condition (3.37 ± 0.76; $F_{1,40} = 4.46$, $P < 0.05$; Cohen's $d = 0.67$). A set of anagrams similar to those described in study 1 were included in this study to test whether avoidance or approach motivations were the driving force for the effect. Results revealed that those in the red versus blue condition responded faster to the avoidance-related anagrams (which indicated an activation of an avoidance motivation) and, subsequently, exhibited a higher score on practicality and appropriateness. In contrast, those in the blue versus red condition responded faster to the approach-related anagrams (which indicated an activation of an approach motivation) and, subsequently, exhibited a higher score on originality and novelty.

Study 5 tested our theorizing in yet another domain, namely persuasive message evaluation. Participants ($n = 161$) evaluated one of two versions of an advertisement for a camera on a computer screen with the background color set to be either red or blue. The two versions of the advertisement were identical except for the visuals (*26*). In one version, the visuals represented specific product details of the camera (e.g., lens) and thus fitted a detail-oriented processing style (*27*). We expected that red, which enhances attention to details, would lead to higher persuasion for this version. The other version included visuals that represented rather remotely related associations (e.g., a road sign, a dining table in a restaurant, and a map), which would require creative thinking to connect all these images to a camera-related theme, e.g., travel (fig. S2). Thus, we expected that blue, which appears to enhance creative cognition, would lead to more persuasion for this version (*27*). Participants evaluated one of the ads on three seven-point items assessing its appeal, favorability, and effectiveness. For the red color computer background screen, participants formed more favorable evaluations when the ad included visuals representing specific product details (4.69 ± 1.26) as opposed to remotely related associations (4.11 ± 1.28; $F_{1,150} = 3.41$, $P < 0.07$; Cohen's $d = 0.42$). In contrast, when the background color was blue, the reverse occurred, i.e., more favorable evaluations emerged when the ad contained visuals representing remotely related associations (4.41 ± 1.47) versus specific product details (3.60 ± 1.59; $F_{1,150} = 6.01$, $P < 0.02$; Cohen's $d = 0.56$) (*8*).

In the last study, we tested whether people are aware of the differential effects of red versus blue color. No color manipulation was done for this study, and all the instructions and the focal task were presented in black color font with white background screen color on computers. Participants ($n = 68$) were told that one of these tasks they would complete requires detailed, careful, and systematic processing of information, and it could be presented to them with either a red or a blue background color. Participants' task was to select one color that they thought would enhance their performance on that task. A sample of the red and blue colors was presented (fig. S3). On the next screen, participants were told that another task in this study would require creative, imaginative, and outside-of-the-box thinking and were asked to select one of the two colors that they thought might enhance their performance on the creative task.

The data revealed that significantly more participants chose the blue (66%) versus red (34%) color when the task was described to be creative [χ^2 (1) = 7.12, $P < 0.01$]. However, interestingly, the same pattern of results emerged when the task was described to be detail-oriented, i.e., more people thought the blue (74%) versus red (26%) background color would enhance their performance even on the detail-oriented task [χ^2 (1) = 15.06, $P < 0.001$] (*8*). These results are consistent with the general belief that people have an overall preference for blue versus red color, although we found that red can be beneficial when the focal task requires detailed attention.

From a series of six studies, using various tasks covering a number of different domains, we demonstrate that red (versus blue) can activate an avoidance (versus approach) motivation and subsequently can enhance performance on detail-oriented (versus creative) cognitive tasks. This research thus offers a reconciliation of the conflicting results reported in the extant literature and advances current research on the effect of color on cognition and behavior [e.g., (*3*)]. More important, our findings offer a wide range of implications for daily human life. What wall color do we pick for an educational facility? What color enhances persuasion in a consumption context? What color enhances creativity in a new product design process? Results from this research suggest that, depending on the nature of the task, different colors might be beneficial. If the task on hand requires people's vigilant attention (e.g., memorizing important information or understanding the side effects of a new drug), then red (or another color that activates an avoidance motivation) might be particularly appropriate. However, if the task calls for creativity and imagination (e.g., designing an art shop, or a new product idea brainstorming session), then blue (or

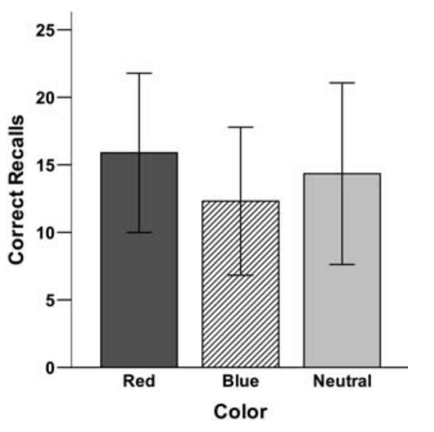

Fig. 2. Total number of correct recalls for the memory task (study 2). $F_{2,100} = 3.15$, $P < 0.05$. Error bars, ±1.00 SD.

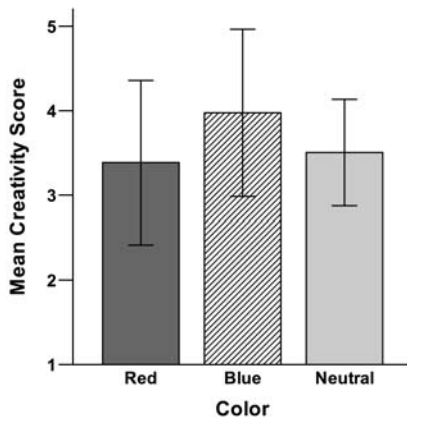

Fig. 3. Mean creativity scores for the brick task (study 2). $F_{2,102} = 4.43$, $P < 0.02$. Error bars, ±1.00 SD.

another color that activates an approach motivation) would be more beneficial.

References and Notes

1. N. Kwallek, C. M. Lewis, *Appl. Ergon.* **21**, 275 (1990).
2. N. J. Stone, *J. Environ. Psychol.* **23**, 63 (2003).
3. A. J. Elliot, M. A. Maier, A. C. Moller, R. Friedman, J. Meinhardt, *J. Exp. Psychol. Gen.* **136**, 154 (2007).
4. S. Soldat, R. C. Sinclair, M. M. Mark, *Soc. Cogn.* **15**, 55 (1997).
5. C. C. Braun, N. C. Silver, *Ergonomics* **38**, 2207 (1995).
6. D. J. Williams, J. M. Noyes, *Theor. Issues Ergon. Sci.* **8**, 1 (2007).
7. N. Kaya, H. H. Epps, *Coll. Stud. J.* **38**, 396 (2004).
8. Materials and methods are available as supporting material on *Science* Online.
9. We recognize that red and blue can also imply other associations. For example, red might connote excitement or femininity, whereas blue might suggest sadness. Although we acknowledge these different associations, we simply suggest that in the cognitive task domain, red is predominately associated with dangers and mistakes and blue with openness and peace. In addition, we note that the same color may have different associations across cultures (*28*). All our studies were run in a North American university. Thus, future research should test whether our results can be generalized to other cultures.
10. R. S. Friedman, J. Förster, *J. Exp. Soc. Psychol.* **38**, 41 (2002).
11. R. S. Friedman, J. Förster, *J. Pers. Soc. Psychol.* **88**, 263 (2005).
12. S. Koch, R. W. Holland, A. van Knippenberg, *Cognition* **109**, 133 (2008).
13. N. Schwarz, in *Handbook of Motivation and Cognition*, E. T. Higgins, R. M. Sorrentino, Eds. (Guilford Press, New York, 1990), pp. 527–561.
14. All studies were run at the University of British Columbia, where students participated in exchange for either course credit (studies 1, 2, 4, 5, and 6) or money (color association study, study 3). The majority of the participants in our studies were raised in Canada, mainland China, and Hong Kong (in decreasing order of prevalence). The participants' ages ranged between 17 and 39 years.
15. E. Thompson, A. Palacios, F. J. Varela, *Behav. Brain Sci.* **15**, 1 (1992).
16. E. T. Higgins, *Am. Psychol.* **52**, 1280 (1997).
17. E. T. Higgins, in *Advances in Experimental Social Psychology*, M. P. Zanna, Ed. (Academic Press, New York, 1998), pp. 1–46.
18. A. J. Elliot, M. V. Coviogton, *Educ. Psychol. Rev.* **13**, 73 (2001).
19. F. I. Craik, J. M. McDowd, *J. Exp. Psychol. Learn. Mem. Cogn.* **13**, 474 (1987).
20. E. Crowe, E. T. Higgins, *Organ. Behav. Hum. Decis. Process.* **69**, 117 (1997).
21. R. S. Friedman, J. Förster, *J. Pers. Soc. Psychol.* **81**, 1001 (2001).
22. S. Kaplan, *J. Environ. Psychol.* **15**, 169 (1995).
23. R. M. Baron, D. A. Kenny, *J. Pers. Soc. Psychol.* **51**, 1173 (1986).
24. V. Griskevicius, R. B. Cialdini, D. T. Kenrick, *J. Pers. Soc. Psychol.* **91**, 63 (2006).
25. C. P. Moreau, D. W. Dahl, *J. Consum. Res.* **32**, 13 (2005).
26. R. J. Zhu, J. Meyers-Levy, *J. Consum. Res.* **34**, 89 (2007).
27. E. T. Higgins, L. C. Idson, A. L. Freitas, S. Spiegel, D. C. Molden, *J. Pers. Soc. Psychol.* **84**, 1140 (2003).
28. M. M. Aslam, *J. Mark. Commun.* **12**, 15 (2006).

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Supporting Online Material

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Self-Sustained Replication of an RNA Enzyme

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An RNA enzyme that catalyzes the RNA-templated joining of RNA was converted to a format whereby two enzymes catalyze each other's synthesis from a total of four oligonucleotide substrates. These cross-replicating RNA enzymes undergo self-sustained exponential amplification in the absence of proteins or other biological materials. Amplification occurs with a doubling time of about 1 hour and can be continued indefinitely. Populations of various cross-replicating enzymes were constructed and allowed to compete for a common pool of substrates, during which recombinant replicators arose and grew to dominate the population. These replicating RNA enzymes can serve as an experimental model of a genetic system. Many such model systems could be constructed, allowing different selective outcomes to be related to the underlying properties of the genetic system.

A long-standing research goal has been to devise a nonbiological system that undergoes replication in a self-sustained manner, brought about by enzymatic machinery that is part of the system being replicated. One way to realize this goal, inspired by the notion of primitive RNA-based life, would be for an RNA enzyme to catalyze the replication of RNA molecules, including the RNA enzyme itself (*1*–*4*). This has now been achieved in a cross-catalytic system involving two RNA enzymes that catalyze each other's synthesis from a total of four component substrates.

The "R3C" RNA enzyme is an RNA ligase that binds two oligonucleotide substrates through Watson-Crick pairing and catalyzes nucleophilic attack of the 3′-hydroxyl of one substrate on the 5′-triphosphate of the other, forming a 3′,5′-phosphodiester and releasing inorganic pyrophosphate (*5*). The R3C ligase was configured to self-replicate by joining two RNA molecules to produce another copy of itself (*6*). This process was inefficient because the substrates formed a nonproductive complex that limited the extent of exponential growth, with a doubling time of about 17 hours and no more than two successive doublings.

The R3C ligase subsequently was converted to a cross-catalytic format (Fig. 1A), whereby a plus-strand RNA enzyme (E) catalyzes the joining of two substrates (A′ and B′) to form a minus-strand enzyme (E′), which in turn catalyzes the joining of two substrates (A and B) to form a new plus-strand enzyme (*7*, *8*). This too was inefficient because of the formation of nonproductive complexes and the slow underlying rate of the two enzymes. The enzymes E and E′ operate with a rate constant of only ~0.03 min^{-1} and a maximum extent of only 10 to 20% (*9*). These rates are about 10 times slower than that of the parental R3C ligase (*5*), and when the two cross-catalytic reactions are carried out within a common mixture the rates are even slower (*7*).

The catalytic properties of the cross-replicating RNA enzymes were improved by the use of in vitro evolution, optimizing the two component reactions in parallel and seeking solutions that would apply to both reactions when conducted in the cross-catalytic format (*9*). The 5′-triphosphate–bearing substrate was joined to the enzyme via a hairpin loop (B′ to E and B to E′), and nucleotides within both the enzyme and the separate 3′-hydroxyl–bearing substrate (A′ and A) were randomized at a frequency of 12% per position. The two resulting populations of molecules were subjected to six rounds of stringent in vitro selection, selecting for their ability to react in progressively shorter times, ranging from 2 hours to 10 ms. Mutagenic polymerase chain reaction was performed after the third round to maintain diversity in the population. After the sixth round, individuals were cloned from both populations and sequenced. There was substantial sequence variability among the clones, but all contained mutations just upstream from the ligation junction that resulted in a G•U wobble pair at this position.

The G•U pair was installed in both enzymes and both 3′-hydroxyl–bearing substrates (Fig. 1B). In the trimolecular reaction (with two separate substrates), the optimized enzymes E and E′ exhibited a rate constant of 1.3 and 0.3 min^{-1} with a maximum extent of 92 and 88%, respectively. The optimized enzymes underwent robust exponential amplification at a constant temperature of 42°C, with more than 25-fold amplification after 5 hours, followed by a leveling off as the supply of substrates became depleted (Fig. 2A). The data fit well to the logistic growth equation $[E]_t = a/(1 + be^{-ct})$, where $[E]_t$ is the concentration of E (or E′) at time t, a is the maximum extent of growth, b is the degree of sigmoidicity, and c is the exponential growth

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