RESEARCH: NEUROSCIENCE

The Biology of Being Frazzled

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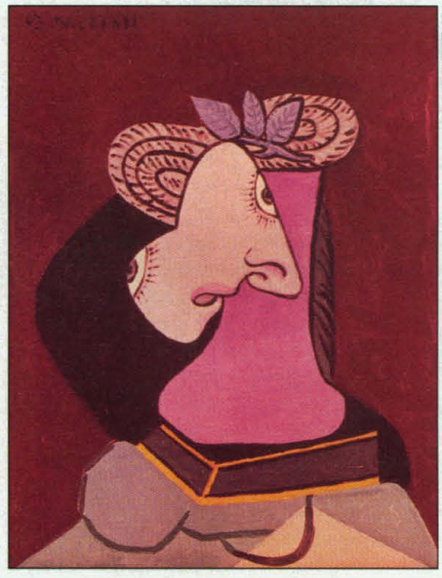
Consider this: You are driving to work, planning an important morning meeting with a colleague and intermittently reminding yourself that you *must* remember to turn left at the traffic light, not right as usual, in order to bring your suit to the cleaners. Suddenly, you find yourself passing an accident—a crowd is gathered around a gruesome scene. The ambulance screams up behind you, and you hurry to get out of the way. You feel your heart quicken and notice that your foot is faster than usual as you step on the brake at the red light. You try to resume planning the morning's meeting, but your thoughts are disorganized now and you lose concentration, distracted by a disk jockey's prattle from the radio. You get to work, the memory of that gruesome scene all too vivid, and berate yourself because you forgot to go to the cleaners.

This scenario captures many of the cognitive changes that occur in response to acute, uncontrollable stress: We become distracted and disorganized, and our working memory abilities worsen, leaving prepotent or habitual responses to control our behavior. Yet our memories of the stressful event are actually better than usual. Neurobiological research can now begin to explain many of these cognitive changes in response to stress. A family of neuromodulators called catecholamines (dopamine, norepinephrine, and epinephrine) are released in the peripheral and central nervous systems during stress. And just as catecholamines "turn on" our heart and muscles and "turn off" the stomach to prepare for fight-or-flight responses during stress, similar opposing actions in the brain may turn on a structure called the amygdala and turn off the prefrontal cortex (a higher cognitive center), allowing posterior cortical and subcortical structures to control our behavior. The amygdala is a phylogenetically older structure in the medial temporal lobe, long known to be essential for the expression of emotion and the formation of associations between stimuli and emotions (*1*). In contrast, the prefrontal cortex expands greatly in primates and permits working memory to guide our behavior, inhibiting inappropriate responses or distractions and allowing us to plan and organize effectively (*2*). High levels of catecholamines exert opposite actions on these brain regions.

Catecholamine stimulation during stress can activate the amygdala and improve memory consolidation (*3*). The pioneering studies of McGaugh, Gold, Tanaka, and others demonstrated that mild to moderate stressors increase norepinephrine release within the amygdala and enhance memory consolidation in rodents. More recently, this work has been translated to humans, in whom the memory of emotionally stressful scenes is associated with activation of the amygdala (*3*) and is mediated via norepinephrine actions at β-adrenergic receptors (*3*). The amygdala is also activated in conditioned fear paradigms, in which a previously neutral stimulus becomes aversive through its association with a stressful stimulus such as an electric shock (*4*). The expression of conditioned fear requires dopamine D1 receptor stimulation in the amygdala in rodents (*5*). Thus, increased release of dopamine and norepinephrine in the amygdala during mild to moderate stress enhances amygdala function. These mechanisms likely contribute to posttraumatic stress disorder in humans (*6*). Catecholamine-induced activation of the amygdala in turn leads to facilitation of declarative memory mediated by hippocampal structures (enhancing memory of the accident scene) and to facilitation of the habit memory functions of the striatum (stepping on the brake faster at the light) (*7*). During stress, the amygdala also induces increased catecholamine release in the prefrontal cortex (*8*). However, in contrast to the facilitative actions in subcortical structures, high levels of catecholamine release in prefrontal cortex result in cognitive dysfunction.



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Lady in a Straw Hat. **P. Picasso, 1936.**

Exposure to mild to moderate uncontrollable stress impairs prefrontal cortical function in humans, monkeys, and rats (*9*). For example, humans exposed to loud noise stress are less able to sustain attention or to inhibit inappropriate responses. As in animal studies, these changes are most evident when the subject feels no control over the stress. In contrast, performance of simple, well-rehearsed tasks can actually be better than usual after stress exposure. Similar results have been seen in studies of rats and monkeys, where stress impairs the spatial working memory functions of the prefrontal cortex but has little effect on the visual discrimination abilities of more posterior cortices. Stress-induced working memory deficits result from increased catecholamine receptor stimulation in the prefrontal cortex and can be ameliorated by agents that prevent catecholamine release or block dopamine receptors (*9*, *10*). Conversely, stress-induced cognitive deficits can be mimicked by infusion of a dopamine D1 receptor agonist in the prefrontal cortex (*11*). Similarly, in electrophysiological studies, large concentrations of D1 agonist abolish the calcium currents that convey signals along dendrites (*12*), effectively "strangling" information transfer from dendrite to soma (*11*). In contrast, the iontophoresis of low levels of D1 receptor antagonists can enhance memory-related neuronal responses in monkeys performing working memory tasks (*13*). These studies emphasize the importance of dopamine D1 receptor actions in taking the prefrontal cortex "off line" during stress. Other neuromodulators may contribute as well [for example, norepinephrine via α1-adrenoceptors (*14*)], ensuring rapid yet reversible loss of prefrontal cortical control over behavior.

This bimodal reaction to stress likely had survival value in evolution: Under stress, the faster, habitual, or instinctual mechanisms regulated by the amygdala, hippocampus, striatum, and posterior cortices would control behavior, and long-lasting memories of aversive stimuli would be enhanced in order to avoid such stimuli in the future. However, in mod-

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ern human society these brain actions may often be maladaptive; now we need prefrontal cortex regulation to act appropriately. These neurochemical changes may explain why the stress of an initial error can cause an athlete to lose concentration and thus lose a competition, or why children in stressful home environments (for example, undergoing divorce) can exhibit behaviors resembling attention deficit hyperactivity disorder, a disorder of prefrontal cortex function. Further research on these important neurochemical mechanisms may help us to elucidate why prefrontal cortical deficits are so prominent in many mental illnesses that are exacerbated by stress (*15*) (affective disorder, schizophrenia) and to develop better treatments for these devastating disorders. And finally, this understanding may allow us to be more compassionate with our own failings in response to life's stressors.

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PROTEIN STRUCTURE

Cytochrome c Oxidase: One Enzyme, Two Mechanisms?

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The cytochrome c oxidases, along with other members of the superfamily of "heme-copper oxidases" (*1*, *2*), are responsible for nearly all aerobic respiration on Earth. These critical enzymes recently provided the occasion for a wonderful—and unexpected—achievement in membrane structural biology. In a single week in the summer of 1995, two independent x-ray structures were reported in *Science* and *Nature*: the 13-subunit enzyme from bovine heart mitochondria (*3*), and the four-subunit enzyme from the soil bacterium *Paracoccus denitrificans* (*4*). Now on page 1723 (*5*) of this issue, Yoshikawa *et al.* report the refinement of the structure of fully oxidized bovine cytochrome c oxidase to 2.30 Å plus the structural changes that occur upon full reduction of the enzyme's metal atoms and upon the binding of ligands, azide, and CO. This major achievement yields a much closer look at this energy-transducing membrane protein and reveals interesting differences between the mammalian and bacterial oxidases that prompt the authors to propose a radically different mechanism of proton pumping for the bovine enzyme.

Cytochrome c oxidase reduces dioxygen (O_2) to water in a way that conserves the considerable free energy made available from this highly favorable reaction (*6*, *7*). This free energy, once harnessed, is then used for a wide variety of energy-requiring biological and biochemical functions, notably ATP synthesis. The oxidase accomplishes this by coupling the redox chemistry (dioxygen reduction to water) to proton pumping, thereby generating transmembrane voltage and proton gradients that supply the proton motive force. For every turnover of the enyzme (dioxygen reduction), eight protons are taken from the inside aqueous compartment, four protons being used to make two molecules of water, and the remaining four protons are pumped to the opposite side of the membrane, about 50 Å away. Understanding how oxidase functions is largely a matter of defining how, when, and where protons move during the catalytic cycle. But knowing the protein architecture of a static structure or set of structures may not be sufficient to deduce mechanism and dynamics.

Cytochrome c oxidase must clearly have more than one extended proton-conducting channel or pathway, but what do such things look like? Net translocation of protons can occur over a long distance through a protein by hopping between pairs of hydrogen-bonded donor and acceptor residues (*8*–*10*). A string of such residues connected by hydrogen bonds can be thought of as a "proton wire" (*9*). Not all hydrogen bond networks can function as a proton wire, because a certain rotational mobility of the component parts of the wire is required. Water is an excellent component of a proton wire (*9*, *10*), but if the internal water molecules are disordered or mobile, or if the x-ray diffraction data are limiting, then these functionally important components of the proton wire will not be apparent. Furthermore, there is no need to have a stable, long-lived continuous hydrogen-bonded chain as a proton wire. Such structures could well be transient, not observed in the static structure, and still carry out their function. In short, finding extended proton-conducting pathways from the x-ray structures is neither sufficient nor necessary to define a "proton wire." Needless to say, this adds some uncertainty to the interpretation of structural data.

Generally, the structures of the bacterial and mammalian oxidases have proven remarkably similar, including significant structural details that have emerged since the original reports (*5*, *11*, *12*). But in their new work, Yoshikawa *et al.* report several differences in their structure of the bovine enzyme. Briefly,

1) The refined structure of the fully oxidized bovine oxidase shows a peroxide molecule at the heme-copper center. This surprising finding will certainly provoke further experiments to demonstrate chemically that the so-called "resting" oxidized form of the enzyme (*13*, *14*) is, in fact, a peroxide adduct. Until spectroscopic and biochemical studies on the crystals can better define which of the several "resting" forms is present, there is no way to know how to relate the unexpected peroxide-containing structure to previous studies of the enzyme in solution. How important this will ultimately be is not clear, because even the authors do not believe that this species is involved in the catalytic cycle.

2) The azide complex of the oxidized bovine enzyme has the same ligation of Cu_B as does the enzyme in the absence of azide. This is in contrast to the "missing" or disordered histidine ligand that was reported in the original structure of the azide adduct of the bacterial oxidase (*4*). The importance of this observation has nothing to do with azide, but rather the implication that the ligands to Cu_B might labile and vary in different states of the enzyme. It has been proposed (*4*, *15*)

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