Stress-Related Noradrenergic Activity Prompts Large-Scale Neural Network Reconfiguration

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Acute stress shifts the brain into a state that fosters rapid defense mechanisms. Stress-related neuromodulators are thought to trigger this change by altering properties of large-scale neural populations throughout the brain. We investigated this brain-state shift in humans. During exposure to a fear-related acute stressor, responsiveness and interconnectivity within a network including cortical (frontoinsular, dorsal anterior cingulate, inferotemporal, and temporoparietal) and subcortical (amygdala, thalamus, hypothalamus, and midbrain) regions increased as a function of stress response magnitudes. β-adrenergic receptor blockade, but not cortisol synthesis inhibition, diminished this increase. Thus, our findings reveal that noradrenergic activation during acute stress results in prolonged coupling within a distributed network that integrates information exchange between regions involved in autonomic-neuroendocrine control and vigilant attentional reorienting.

Acute stress alters the way our brain functions. This brain-state shift can be understood as a strategic reallocation of resources to functions that are vital when survival is at stake: It sharpens our senses, creates a state of fearful arousal (*1, 2*), and strengthens our memories of stressful experiences (*3–5*), but impairs our capacity for slow deliberation (*6, 7*).

Animal research into the acute stress response has delineated a chain of neurochemical events triggering the release of various hormones and neurotransmitters (*1, 8*). Acting as neuromodulators, these alter cellular properties of large-scale neuronal populations throughout the brain. Activation of the hypothalamic-pituitary-adrenal (HPA) axis, resulting in increased systemic release of corticosteroids, is the hallmark of the stress response. However, a host of central changes in neuropeptide and monamine release plays a key role at shorter time scales (*1, 5*). For instance, acute stress elevates tonic firing rates in the locus coeruleus (LC), the primary source of noradrenaline in the forebrain (*9–11*), and corticosteroid effects in multiple brain regions depend on concomitant noradrenergic activation (*4*). We therefore hypothesized that stress-related neuromodulators, in particular noradrenaline, trigger brain-state alterations by reorganizing neural activity within large-scale neuronal systems (*12*).

We tested this hypothesis in two experiments using model-free neuroimaging analyses that allow the quantification of state changes during "real-world" experiences (*13*). To induce the intended change in a scanner environment while optimally preserving dynamic sensory and affective qualities of real-world threatening events, we exposed participants to highly aversive cinematographic material (*6*) presented uninterrupted during blood oxygenation level–dependent functional magnetic resonance imaging (BOLD-fMRI). In experiment 1, participants (80 healthy volunteers) also saw a neutral movie matched for audiovisual characteristics (table S1) in a separate counterbalanced session. Physiological and psychological stress measures were obtained around and during scanning. Exposure to the aversive movie triggered elevated salivary cortisol [$F(1, 79) = 4.93$, $P = 0.029$, partial eta-squared ($P\eta^2$) = 0.06], salivary alpha amylase [marker of (nor)adrenergic activity; $F(1, 79) = 5.61$, $P = 0.02$, $P\eta^2 = 0.07$], and heart rate [$F(1, 78) = 44.20$, $P < 0.001$, $P\eta^2 = 0.36$], and increased subjective negative affect [$F(1, 79) = 23.37$, $P < 0.001$, $P\eta^2 = 0.23$].

We first identified brain regions that responded preferentially to the aversive movie. Instead of using a pre-specified model that imposes restrictions on the temporal shape of the response that can be detected, we capitalized on the fact that regional activation can be inferred from temporal correlations across participants [fig. S1 (*13*)]. We observed strong intersubject correlations (ISCs) mainly, but not exclusively, in sensory regions during both movies (Fig. 1, and B, and

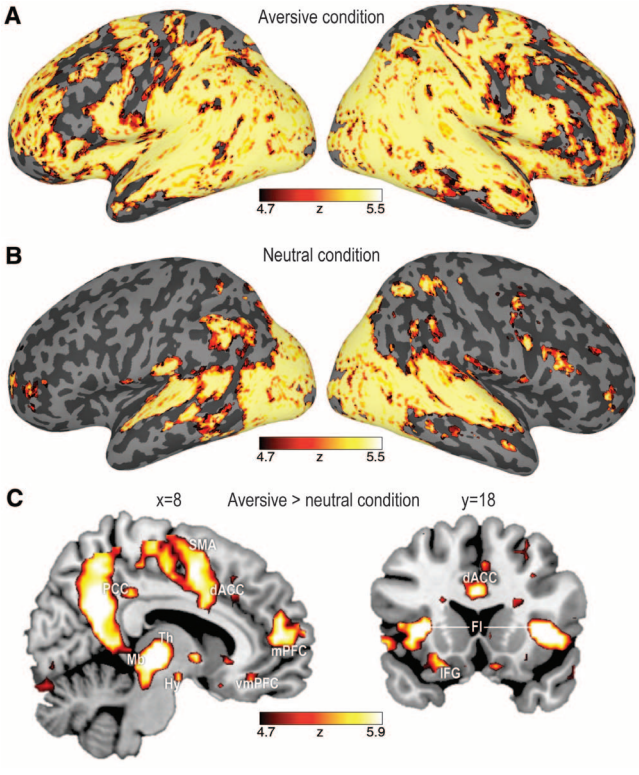

Fig. 1. ISCs. Maps are thresholded at $P < 0.05$, whole-brain FWE-corrected, and overlaid onto cortical surface renderings (**A** and **B**) and a canonical structural MRI (**C**). FI, frontoinsular cortex; SMA; supplementary motor area; PCC, posterior cingulate cortex; (v)mPFC, (ventro)mPFC; IFG, inferior frontal gyrus; Th, thalamus; Mb, midbrain; Hy, hypothalamus.

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table S2). A contrast between both conditions' ISC maps produced by nonparametric permutation tests [$P < 0.05$, whole-brain family-wise error (FWE)–corrected (*14*)] revealed relatively few ISC differences in early visual regions. However, we found increased ISC for the aversive movie in regions (table S3 and Fig. 1C) shown to respond consistently to salient stimuli in meta-analyses of conventional model-based fMRI studies (*15, 16*). Among these are regions associated with interoception and autonomic-neuroendocrine control [frontoinsular cortex, dorsal anterior cingulate cortex (dACC), medial prefrontal cortex (mPFC), and amygdala (*17–19*)], peripheral stress effector systems and catecholaminergic signaling [midbrain and hypothalamic regions (*8, 15*)], and sensory and attentional (re)orienting [thalamus, and inferotemporal and temporoparietal regions (*20*)]. A similar set of regions forms an intrinsic connectivity network (ICN) in the resting brain that has been proposed to process salience by integrating affective-homeostatic with sensory-attentional information (*21*). The temporal correlations across participants found here, however, provide no information about functional connectivity, because different regions may respond to different aspects of the movie and therefore display uncorrelated time courses.

To test for functional connectivity, we used multisession tensorial probabilistic independent component analysis (ICA). We decomposed fMRI data into time courses, spatial maps, and subject modes, which represent signal variation of each IC over time, space, and participants, respectively [see supporting online material (SOM) (*22*)]. ICA for the aversive condition yielded 18 IC maps (fig. S2), which represent spatially dissociable signal fluctuations originating from separable large-scale neural ensembles (or nuisance sources). Using objective template matching (table S5), we subsequently identified the IC map with the strongest overlap with the ISC contrast map (aversive > control; Fig. 2 and fig. S3). The thereby selected IC map for the aversive condition contained all regions mentioned in the previous paragraph except the mPFC (see Fig. 2 and table S4 for all coactivated regions). Furthermore, template matching onto a map of the aforementioned salience-processing ICN, kindly provided by the authors of (*21*), yielded the same IC map (table S5). In the remainder, we therefore refer to the selected IC map as the salience network (*21*). The mPFC appears in another IC map alongside the posterior cingulate cortex, suggesting that these regions form part of another neural system [the default mode network (*12*)].

To investigate whether functional connectivity strength within the salience network was associated with stress measures, we used compound measures resulting from ICA decomposition (*22*). Network strength correlated positively with cortisol [Spearman's $\rho(78) = 0.23$, $P = 0.037$], alpha amylase [$\rho(78) = 0.28$, $P = 0.012$], and negative affect change [$\rho(78) = 0.25$, $P = 0.026$], but not heart rate change [$\rho(78) = -0.06$, n.s.].

Our findings agree with theories that postulate a dual architecture of cortical attentional control networks. In addition to a dorsal fronto-parietal network involved in regulating attention in focal tasks (*23*), these theories implicate a ventral attention network that differs little in topology from the network identified here in reorienting attention away from focal tasks (*20*) and the maintenance of tonic alertness (*24*). Spontaneous activity in this network has moreover been associated with electroencephalographic signatures of alertness (*25*).

A pivotal question following from these observations is to what extent stress-related neuromodulators such as noradrenaline and cortisol drive this network reorganization. To address this, we performed a pharmacological experiment (experiment 2) implementing a three-armed double-blind between-participants design. Sixty participants received either propranolol (40 mg), a β-adrenergic receptor blocker; metyrapone (750 mg given twice), a cortisol synthesis blocker; or a placebo (Fig. 3). Stress induction procedures were extended with a threat of mild electrical shock to increase effectiveness in raising cortisol but were otherwise identical to experiment 1 (SOM).

We observed robust cortisol responses to stress after the placebo [$F(1, 19) = 8.67$, $P = 0.008$, $P\eta^2 = 0.31$] and propranolol [$F(1, 19) = 11.93$, $P = 0.003$, $P\eta^2 = 0.39$], but not after metyrapone ($F < 1$). Metyrapone lowered cortisol throughout testing [$F(1, 38) = 11.60$, $P = 0.002$, $P\eta^2 = 0.23$]. Conversely, propranolol selectively lowered alpha amylase throughout testing [$F(1, 37) = 9.10$, $P = 0.005$, $P\eta^2 = 0.20$; metyrapone effect: $F < 1$], and lowered heart rate [$F(1, 35) = 29.11$, $P < 0.001$, $P\eta^2 = 0.45$; metyrapone effect: $F(1, 36) = 1.7$, n.s.]. Neither drug affected subjective negative affect ($F < 1$). Thus, as intended, propranolol and metyrapone selectively affected (peripheral) noradrenergic and glucocorticoid measures, respectively (Fig. 3).

ICA (fig. S4) and template matching of IC maps between experiments 1 and 2 closely reproduced the salience network IC map (Fig. 4A

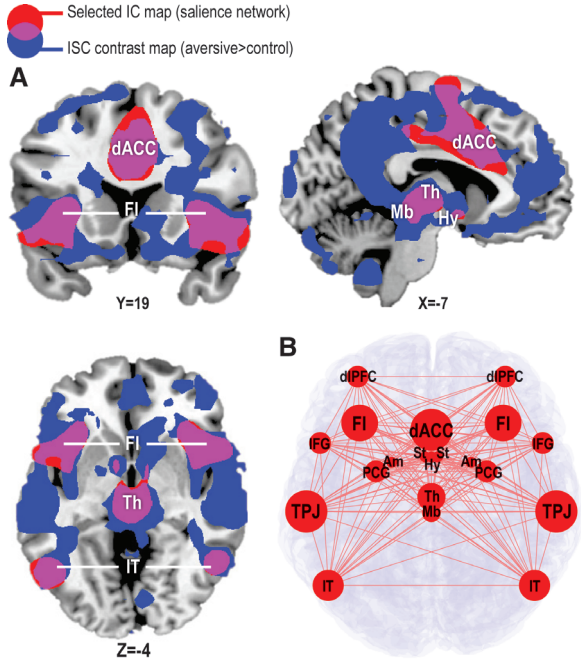

Fig. 2. Regions making up the selected IC map (salience network; red). (**A**) Overlap (pink) with ISC contrast map (blue; $P < 0.001$). (**B**) Schematic overview of suprathreshold clusters and relative sizes. IT, inferotemporal cortex; TPJ, temporoparietal junction; Am, amygdala; PCG, precentral gyrus; dlPFC, dorsolateral PFC; St, striatum (caudate/pallidum).

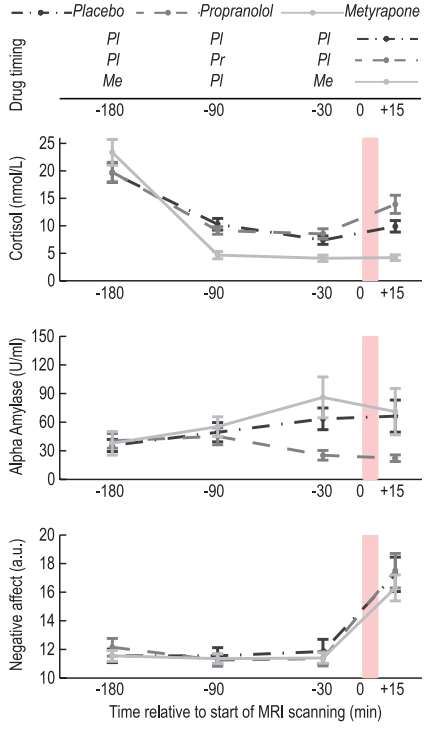

Fig. 3. Timing and effects (±SEM) of drug administration. Shaded red bars indicate the stressor (average time: 12:30 p.m.). Pl, placebo; Pr, propranolol; Me, metyrapone.

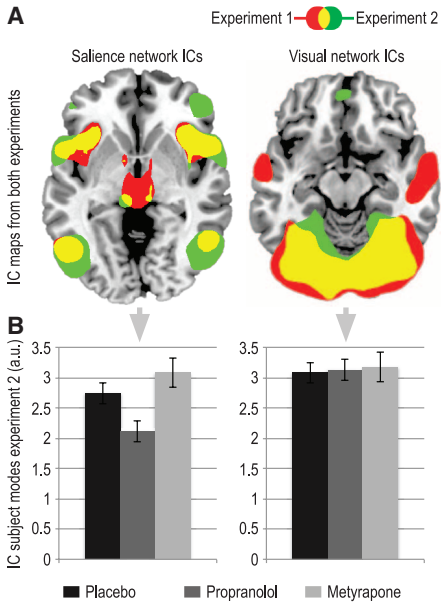

Fig. 4. Drug effects on functional connectivity within salience and visual (control) network ICs. (**A**) Overlap between the IC maps from both experiments ($P < 0.001$). (**B**) Functional connectivity strength (±SEM) within both ICs for drug conditions (experiment 2). a.u., arbitrary units.

and table S5). We investigated drug effects on functional connectivity strength within this network in comparison with a visual network as a control for specificity. A 3 (drug) × 2 (IC) analysis of variance yielded a drug-by-IC interaction [$F(2, 57) = 3.46$, $P = 0.038$, $P\eta^2 = 0.11$]. Further testing revealed a drug main effect on the salience [$F(2, 57) = 3.19$, $P = 0.049$, $P\eta^2 = 0.10$] but not the visual ($F < 1$, n.s.) network. A planned contrast showed that this effect was carried by a reduction in the propranolol group as compared to the other groups [$F(1, 57) = 5.61$, $P = 0.021$, $P\eta^2 = 0.09$]. Finally, directed one-tailed t tests demonstrated that propranolol reduced network strength relative to both the placebo [$t(38) = 1.64$, $P = 0.054$] and metyrapone [$t(38) = 2.41$, $P = 0.011$] groups.

This finding concurs with theoretical frameworks of LC function, which ascribe attentional reorienting functions to cortical noradrenergic projections that parallel those proposed for cortical components of the salience network (*20*). Animal studies have shown that LC neurons exhibit two distinct functional modes for regulating sensory gain (*26*). In mildly aroused states that are optimal for focal task performance, the LC responds phasically to task-relevant stimuli (*9*), engaging α-2A receptors that strengthen top-down dorsolateral PFC regulation of attention (*7*). Under stress, however, LC neurons shift to tonically elevated firing rates associated with distractibility and hypervigilance (*10*). High tonic firing releases large concentrations of norepinephrine, which engages lower-affinity β-adrenergic receptors that impair top-down attentional control but enhance thalamic and sensory functions (*7*). Thus, besides effects on memory (*3, 4*), a putative function of these neuromodulatory signals is to send interrupt signals to active functional networks (*27*), causing disengagement from current task sets (*9*) and promoting fast adaptation by rearranging network activity (*11*). Our findings establish a causal link between stress-induced noradrenergic activity and activation of the salience network (*20*).

Although functional connectivity within the salience network correlated with cortisol increases (experiment 1), our finding that cortisol blockade had no effect suggests that cortisol elevation is not necessary for this network reorganization to occur. It has been suggested that corticosteroids act through mineralocorticoid receptors to promote vigilance in immediate response to stress (*1*). However, recent studies show that exogenous cortisol reduces phobic fear (*28*) and amygdala responsiveness (*29*), pointing toward a role for cortisol in preventing overshoot and down-regulation of stress responses. Nonetheless, we cannot exclude the possibility that with different timing or stronger elevations of cortisol, interactive or additive effects may occur (*4*).

We have shown that noradrenergic neuromodulatory activity in the early phase of the stress response drives a reallocation of neural resources toward a distributed network of regions involved in attentional reorienting, vigilant perceptual intake, and autonomic-neuroendocrine control.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/334/6059/1151/DC1
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