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21. *Xenopus* egg or oocyte extracts were prepared as described (*4*) and immunoprecipitated with rabbit polyclonal antiserum (1913.2) to the COOH-terminal Xp42 peptide KELIFEETARFQPGY (*29*). For immune complex kinase assays, immunoprecipitates were incubated in 30 μl of KB [20 mM Hepes (pH 7.5), 2 mM dithiothreitol (DTT), 1 mM $MgCl_2$, 1 mM EGTA, 1 mM phenylmethylsulfonyl fluoride (PMSF), and 2 mM Na_3VO_4] with MBP (1 mg/ml) (Sigma) and 5 μCi of [γ-^{32}P]ATP (3000 Ci/mmol, Du Pont Biotechnology Systems) for 15 min at 30°C before electrophoresis. For phosphatase treatment, immunoprecipitates were incubated in phosphatase buffer [25 mM imidizole (pH 7.5), 0.1% 2-mercaptoethanol, and bovine serum albumin (0.1 mg/ml)] with the indicated phosphatase for 20 min at 30°C and washed twice in KB.
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25. *Xenopus* egg extract was labeled in vitro with [^{32}P]orthophosphate [J. A. Cooper, *Mol. Cell. Biol.* **9**, 3143 (1989)] and immunoprecipitated with antiserum 1913.2. The immunoprecipitates were run on SDS gels, and Xp42 was excised from the gel and digested with trypsin. The tryptic phosphopeptides were separated in two dimensions on thin-layer chromatography plates by electrophoresis at pH 8.9 (anode at left) and ascending chromatography with buffer II [A. Kazlauskas and J. A. Cooper, *Cell* **58**, 1121 (1989)].
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27. Xp42 cDNA was subcloned into plasmid pSP64A (Promega). Site-directed mutagenesis of Xp42 cDNA was done as described [T. A. Kunkel, J. D. Roberts, R. A. Zabour, *Methods Enzymol.* **154**, 367 (1987)]. RNA was transcribed in vitro with SP6 RNA polymerase (Promega). [^{35}S]methionine (1100 Ci/mmol, Tran^{35}S label, ICN Biomedicals) was dried and resuspended in a solution of RNA (0.5 mg/ml) at 38 μCi/μl. This solution (30 to 50 nl) was injected into the cytosol of Stage VI oocytes. The oocytes were incubated in 50% L-15 medium (Gibco) with gentamycin (0.05 μl/ml) at 15°C for 2 days. On day 3, progesterone (2 μg/ml) was added, and incubation was continued for 16 hours. Extracts were prepared by homogenizing groups of 20 to 35 oocytes in 20 mM Hepes (pH 7.5), 2 mM DTT, 2 mM $MgCl_2$, 1 mM EGTA, 1 mM PMSF, 2 mM Na_3VO_4, and 0.05 μM microcystin (Calbiochem), and insoluble debris was removed by centrifugation.
28. J. Posada and J. A. Cooper, unpublished results.
29. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
30. A bacterial protein containing glutathione transferase (GST) fused to the NH_2-terminus of Myc (GST-Myc) (AMRAD) was prepared according to the manufacturer's instructions. This protein contains a site phosphorylated by MAP kinase [E. Alvarez *et al.*, *J. Biol. Chem.* **266**, 15277 (1991)].
31. Egg extract (2 mg of protein) was diluted in column buffer [20 mM Hepes (pH 7.5), 5 mM $MgCl_2$, 1 mM EGTA, 5 mM 2-mercaptoethanol, 1 mM PMSF, 2 mM Na_3VO_4, and 0.5 μM microcystin] containing 0.1% 3-[(3-cholamidopropyl)dimethylammonio]-1-propane sulfonate (CHAPS) and was applied to a 1-ml DE-52 (Whatman, Madistone Kent, England) anion exchange column. The column was washed with one bed volume and eluted with 0.1 M and 0.3 M NaCl in column buffer (1 ml of each). The fractions were concentrated in centrifugal concentrators (Amicon), made 5 mM in ATP, and mixed with 9E10 immunoprecipitates containing ^{35}S-labeled methionine Xp42-MT. The mixtures were incubated at 30°C for 30 min and washed, and immune complex kinase assays carried out in the presence of GST-Myc and [γ-^{32}P]ATP.
32. We thank C. Diltz and E. Fischer for CD45, T. Haystead for PP2A, B. Blackwood for GST-Myc, M. Roth for Myc tag reagents, and A. Kashishian for technical assistance. Supported by grants from the NIH (CA-28151 and CA-08860).

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Technical Comment

Localization of Responses to Pain in Human Cerebral Cortex

J. D. Talbot *et al.* (*1*) found significantly increased cerebral blood flow (CBF) in the contralateral anterior cingulate cortex (area 24) in response to thermal pain in normal volunteers. We have confirmed this result (*2*) using different methods of statistical analysis, but we also found increased CBF in the contralateral thalamus ($z = 4.193$, $P < 0.01$ for the center of both areas). We conclude that these two areas are likely to be the main sites in the brain where the "suffering" component of pain is experienced. The results of cingulumotomy on patients with intractable pain suggest that both attentional and emotional components of pain may be processed in the anterior cingulate cortex (*3*).

In contrast to the findings in (*1*), we did not see any evidence of increased CBF in the primary or secondary somatosensory cortices in response to an increasing intensity of nonpainful heat or to a painful heat stimulus. The explanation for this different result may well be that we stimulated the same area of the back of the hand to exclude the tactile and positional components of the stimulus, whereas Talbot *et al.* carefully moved the site of stimulation around the forearm. It seems likely that, in the latter procedure, the variable tactile components, and the subject's variable attention to the positional components of the stimulus, produced increased CBF in the primary and secondary sensory cortices.

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Response: Although our finding of activation of the anterior cingulate cortex in response to pain has been confirmed by Jones *et al.* (*1*), our interpretation of this activation differs from their "suffering" hypothesis. Psychophysical studies (*2, 3*) have demonstrated that phasic noxious heat stimuli evoke pain sensations with a low affective component. On the basis of anatomical (*4*), neurophysiological (*5*), and human stimulation (*6*) studies, we postulate that (exclusively contralateral) cingulate activation may indicate a direct encoding of stimulus intensity rather than any substantial "suffering" experienced by the subjects. Our views are not completely at odds with those of Jones *et al.*, as we presume that the encoding of pain intensity is a major factor in the regulation of emotional and autonomic responses. Nevertheless, "suffering" is not likely to be

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the most prominent perceptual consequence of the brief, tolerable heat pulses used in our experiments.

We consider the contralateral thalamic activation demonstrated by Jones *et al.* (*1*) to be an indication of activity in somatosensory pathways signaling noxious stimulus intensity rather than an indication of "where the 'suffering' component of pain is experienced." Activation of presumed affective pathways would likely be *bi*lateral in both thalamic and cortical areas [see references in (*5*)] and has been demonstrated in imaging studies of anticipatory anxiety (*7*). We did not report data about activation of thalamus because some of our subjects did not receive scans in this region.

The suggestion of Jones *et al.* that the activation we observed in primary and secondary somatosensory cortices resulted from variable tactile or attentional components related to stimulus presentation is plausible, but not the most parsimonious explanation. Reanalysis of our data, with refinements in the stereotactic averaging across subjects, revealed that the pain-related activation in primary somatosensory cortex was stronger than we had originally calculated [$P < 0.002$; change-distribution analysis (*8*)]. In both conditions within our substraction pair—noxious heat and innocuous warm control—the same spots of skin were touched, in the same sequence, with the same thermode, for identical durations.

Attention-related activation is a plausible explanation for *any* observed changes in blood flow related to noxious stimuli, as a change in attentional state is practically an obligatory consequence of the perception of pain. We attempted to control for this confounding variable by requiring subjects to attend to and rate numerically the mean intensity of the stimuli presented during each experimental condition. Anatomical data support the existence in primates of distinct spinothalamocortical pathways that relay nociceptive information to the primary somatosensory cortex (*9*). Neurophysiologic data from anesthetized and awake primates demonstrate that neurons within the primary somatosensory cortex encode both temporal and intensity components of noxious thermal stimuli. Moreover, the nociceptive responses of these neurons do not depend on innocuous tactile or attentional variables (*10, 11*).

Jones *et al.* (*1*) used stimulation parameters substantially smaller than ours. Their 46.4°C stimuli only approximate the threshold for nociceptive activity in primary somatosensory neurons (*11*) and for pain perception (*2, 3, 12*). Their application of noxious stimuli during only one third of the scan time probably limited the accumulation of radioactivity. Their use of only one stimulation site did not take advantage of the spatial summation observed with multiple sites (*3*). Any one of these differences could have reduced the likelihood of observing pain-related activation in the somatosensory cortex.

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