Previous attempts to delineate features important for binding at the opiate receptor (*5–7*) have been based primarily on the effect of substitution on activity. Our study provides structural data that can be used as a basis for understanding the activity of various analogs. For example, the observed ϕ and ψ torsion angles of both Gly residues describe a conformation that is forbidden to L-amino acids, and therefore the activity of the D-Ala^2 analog (*7, 17*) should not be unexpected since this amino acid can easily adopt the conformation of Gly^2 and at the same time provide protection against proteolysis. The activity of the *N*-methyl-Tyr^1 (*7*) analog can also be understood on the basis of the solid state results since the primary amino group has sufficient space around it for one or two methyl groups without disrupting either the hydrogen bonding scheme or the overall conformation. However, the active D-Ala^2, *N*-methyl-Phe^4 (*17*) analog must have a different conformation from that observed in our study because of the bulk of the additional methyl group and the loss of one of the hydrogen bonds.

Other investigations have enumerated chemical features important for activity at the opiate receptor (*5–7, 17*). Our study provides a rational basis for the delineation of five regions (Fig. 3) which are important, but not all necessary, for opiate activity: (i) a phenyl hydroxyl group, attached to the A ring of morphine, analogous to the Tyr hydroxyl of enkephalin; (ii) an amino nitrogen, the tertiary nitrogen of morphine, analogous to the primary amino group of enkephalin [H-Tyr]; (iii) a hydrophobic region, the C(7)–C(8) face of morphine located in the same region as the Leu side chain and analogous Met side chain in [Met^5]enkephalin; (iv) a hydrophilic group in the region of the O(6) of morphine, which corresponds to the carboxyl terminus of enkephalin; and (v) a phenyl ring, which is missing in morphine but identified as the F ring in a number of more potent opiate drugs, and which corresponds to the relatively unconstrained Phe side chain of enkephalin.

Thus, the peptide backbone of enkephalin stabilized by two intramolecular hydrogen bonds provides the rigid frame to which the side chains are attached in a specific spatial relationship. Optimal binding to the opiate receptor is accomplished by minor adjustments to the orientations of these side chains by rotations about the $C(\alpha)$–$C(\beta)$ and $C(\beta)$–$C(\gamma)$ bonds.

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Pupillometric Signs of Brain Activation Vary with Level of Cognitive Processing

Abstract. *The idea that hierarchically higher brain processes require greater amounts of central nervous system vigilance or activation for their execution was tested in two experiments measuring pupillary dilation during the decision interval of a hierarchically structured letter-matching task. Larger dilations indicative of increased activation were observed for letter pairs requiring higher levels of processing.*

Hughlings Jackson (*1*) in 1884 proposed that functional processes in the human nervous system are hierarchically organized, with the higher levels being increasingly unconstrained or plastic, complex, and voluntary as opposed to automatic. Jackson recognized that factors that reduce central nervous system (CNS) vigilance selectively affect the highest levels of integration, an idea which Head later extended in his writings (*2*). By vigilance was meant the general state of nervous system activation that is now thought to be reflected as electrocortical desynchronization and autonomic arousal (*3*).

These early investigations studied the level of integration that may be accomplished when the capacity of the nervous system to sustain a normal state of activation has been impaired by either injury, disease, or the effects of drugs (*2*). A modern example of this experimental approach is the discovery that the aphagia and adipsia following lateral hypothalamic lesions are due in large part to a disruption of endogenous activation systems and that with recovery of these systems the hierarchically organized processes governing feeding and drinking return in a Jacksonian sequence of increasing complexity (*4*). However, it appears that the level of activation in the normal organism is not fixed, but varies from moment to moment and task to task according to the processing demands placed upon the nervous system (*5*).

We now report the results of two experiments that suggest that hierarchically organized cognitive processes vary in the degree to which CNS activation is mobilized during their execution. The cognitive task employed was letter matching, in which a pair of visually presented upper case letters, lower case letters, or one of each are judged by an observer to be the same or different (*6*). If a name criterion is employed as in the first experiment, letter pairs may be judged to be the same if they are orthographically identical (for example, AA or aa) or differ in orthography but share the same name (for example, Aa). Only the physical features of the former pair need be processed before a judgment can be reached, whereas the stimuli must be processed at the higher level of naming for the latter type of pair. When a category criterion is used, as in the second experiment, letters are judged same if they belong to a common category, vowels or consonants. In this case a third

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type of same judgment is introduced in which a physical comparison is made at the lowest level, then the names are extracted, and then membership in a common category is judged (for example, Ae or BR). Posner and Mitchell (*7*) have presented convincing arguments from reaction-time data that the processes of feature analysis, name code extraction, and category membership testing are hierarchically organized. In Jackson's (*1*) terms, matches made at the physical, name, and category levels are ordered by increasing plasticity and complexity and decreasing automaticity of the central processes by which they are mediated. Extending Head's reasoning, one would expect that decision processes executed at different levels of this hierarchy differ systematically in the degree to which they demand activation.

Of the several available methods for measuring activation, perhaps the most sensitive and reliable is the measurement of sympathetic and parasympathetic activity as reflected in pupillary diameter (*8*). Pupillary dilations indicating momentary increases in CNS activation as a function of processing load (*9*) have been reported for short-term memory (*10*), problem-solving (*11*), and other complex information-processing tasks (*12*). Further, pupillometric measures have shown a striking correspondence to electrophysiological indices of activation in a long series of studies of corticoreticular interactions (*13*). The pupillary dilations indicative of CNS arousal may be mediated by either increased forebrain inhibition of the Edinger-Westphal nuclei or increased sympathetic discharge (*14*). Thus pupillometric methods appear well suited to the measurement of short-term activation changes in man that occur in information-processing tasks.

Sixteen undergraduates served as observers in the first experiment using a name criterion for judging 144 letter pairs that were presented tachistoscopically on a computer-controlled cathode-ray tube display. Random dot patterns preceded and followed presentation of the letter pair so that the illumination level of the display was constant at all times. The display field subtended a visual angle of 0.5° and was viewed at a distance of 4 m. Observers initiated each trial with a button press, which was followed 1 second later by the presentation of the letter pair for 100 msec. Two seconds after stimulus onset, a response cue was displayed and the observer indicated his judgment by pressing one of two microswitches. Pupillary diameter was measured with a Whittaker 1053 TV pupillometer and was recorded at 20-msec intervals between trial initiation and response cue presentation. Room illumination was 18 mL.

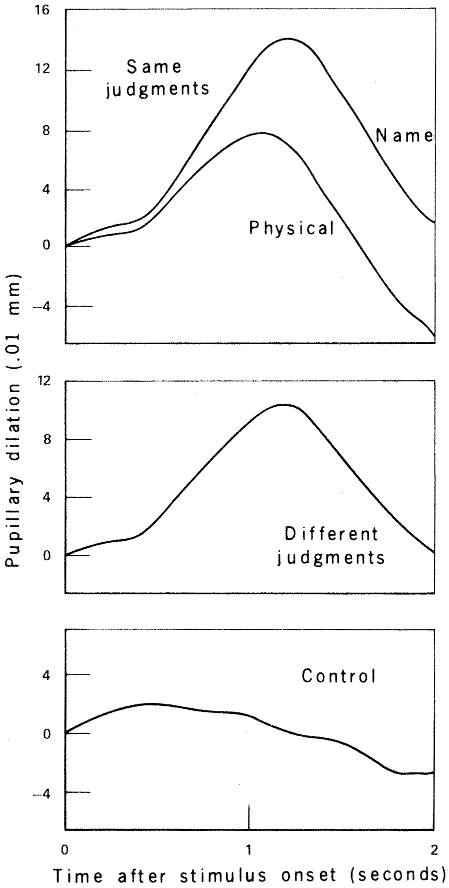


Fig. 1. Averaged evoked pupillary responses for same, different, and control judgments for a group of 16 subjects in a letter-matching task using a name-level criterion.

Six letters (AEIBRH) were employed and displayed in either upper or lower case. Of the 144 trials, 36 letter pairs were physically identical (PI), 36 were identical at the level of naming (NI), and 72 were of different (D) names.

Following the main experimental session, pupillometric measurements were taken in a series of 16 control trials, in which the stimuli were always the letter pair XX and the subject was instructed to press the "same" switch following the response cue. Thus encoding and decision processes were not required in the control series.

Pupillary and behavioral data from each trial were stored on magnetic disks for later analysis. Artifact detection was performed blindly with respect to stimulus type and response correctness by visual inspection of individual evoked pupillary responses. All trials containing major artifacts were discarded. Trials with small artifacts occurring in noncritical periods were corrected by linear interpolation. This procedure rejected approximately 5 percent of all trials in each of the stimulus categories (PI = 5.4 percent, NI = 4.7 percent, and D = 4.6 percent).

Under these conditions, subjects made few errors of judgment, but the percentage of errors differed significantly among the stimulus categories (PI = 1.4 percent, NI = 10.4 percent, and D = 1.7 percent; $F = 14.09$, d.f. = 2,30, $P < .001$). More errors occurred for letter pairs sharing only the same name than for physically identical or different letter pairs.

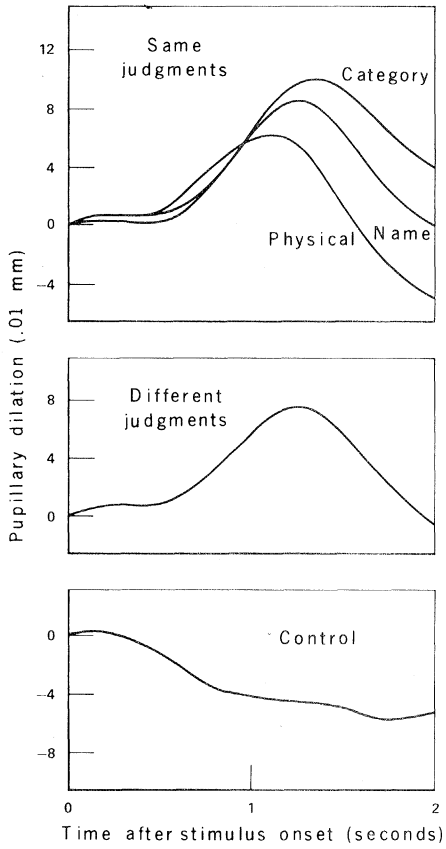
Performance of the letter-classification task was associated with increased pupillary dilation. For each subject separate averaged evoked pupillary responses were computed for all artifact-free control and errorless experimental trials. The difference between prestimulus pupillary diameter and pupillary diameter averaged during the decision interval between stimulus presentation and response was significantly larger in experimental than in control trials ($t = 2.47$, d.f. = 15, $P < .02$). Thus the pupillometric measure appears sensitive to the encoding and decision components of the letter-classification task.

The degree of dilation observed in the decision interval for correct same judgments depended upon level of processing required to match the letters of the stimulus pair. Figure 1 presents the group averaged evoked pupillary responses for all experimental conditions. For the same judgments, these differences are most apparent in the second half of the decision interval. The mean dilation in the 1-second period preceding the response cue differed significantly between the two levels of same judgments ($t = 2.68$, d.f. = 15, $P < .05$). The dilation observed for correct different judgments was of intermediate amplitude.

A more stringent test of the relationship between processing level and task-induced activation may be obtained by employing a more elaborated hierarchical processing structure in the letter-matching task. For this reason a second experiment was performed, with a category-level criterion for judgment, in which the subject responds "same" if both letters are members of the same category, either vowels or consonants. In the second experiment 24 letter pairs were physically identical, 24 were identical at the level of naming, and 24 differed in name but were members of a common category (CI). Seventy-two letter pairs differed in both name and category membership. Sixteen naive individuals served as subjects. In all other respects, the methods of the two experiments were identical.

The results of this second experiment

Fig. 2. Averaged evoked pupillary responses for same, different, and control judgments for a group of 16 subjects in a second experiment using a category-level criterion.



confirmed in more detail the relations between the level of hierarchical processing and task-induced activation. As in the first experiment, subjects made few errors of classification, but the percent of judgment errors differed significantly among the experimental conditions (PI = 0.2 percent, NI = 2.9 percent, CI = 8.1 percent, and D = 0.5 percent; $F = 4.24$, d.f. = 3,45, $P < .025$).

The averaged evoked pupillary responses for the second experiment are shown in Fig. 2. As in the previous experiment, few trials were rejected for artifacts in the pupillometric data and these rejections were not selectively distributed across stimulus conditions (PI = 4.4 percent, NI = 4.6 percent, CI = 5.0 percent, and D = 4.2 percent). And as before, pupillary dilation during the decision interval was significantly larger on experimental than on control trials ($t = 3.72$, d.f. = 14, $P < .01$, the control trial data of one subject being lost in computer malfunction).

As might be apparent from Fig. 2, highly reliable differences are present in the averaged evoked pupillary responses for correct same judgments as a function of the level to which the letter pair must be processed before reaching a correct decision of identity. For the three types of letter pairs that may be judged "same" by the category criterion, the averaged pupillary dilation in the 1-second interval preceding response cue onset is smallest for physically identical letter pairs and largest for categorically identical pairs of different names. These differences in dilation amplitude are highly significant ($F = 6.46$, d.f. = 2,30, $P < .005$). Further, the latency to peak pupillary response is significantly larger for letter pairs requiring higher levels of processing (PI = 1.145 seconds, NI = 1.306 seconds, and CI = 1.345 seconds; $F = 6.73$, d.f. = 2,30, $P < .005$). Thus less-automatic processing of same letter pairs within this hierarchically organized cognitive system (*7*) appears to be associated with larger amounts of activation for greater periods of time.

In both experiments, the amplitude of the averaged evoked pupillary response for correct different judgments was intermediate between those observed for the simplest and most complex same judgments. This result is in agreement with reaction-time data obtained with simultaneously presented letter pairs (*7, 15*). One interpretation of both sets of findings is that some different letter pairs can be judged to be different on the basis of physical comparison, whereas others require full processing before a correct classification can be made. The apparent heterogeneity of the different judgments for simultaneously presented letter pairs therefore does not cloud the orderly relations observed between processing depth and task-induced activation observed for correct same judgments.

The use of letter matching as a procedure for the investigation of analysis and decision processes carried out at different levels of a hierarchically organized cognitive system coupled with the use of pupillometric measurements to assess momentary shifts in centrally regulated autonomic activation provides a unique opportunity to test the Jacksonian hypothesis that more complex cognitive processes require a greater level of activation or "vigilance" for their successful execution. The data from both experiments lend support to this hypothesis. They suggest a pattern of nervous system organization in which the presumably forebrain mechanisms that specifically mediate complex cognitive processes exert reasonably direct, short-latency control of brainstem activation systems to provide the momentary level of central activation required. However reasonable such an interpretation might be, it is not without its difficulties. First, despite several decades of serious investigation, the mechanism by which brainstem activation systems affect the efficiency of cortical information processing remains puzzling (*16*). Second, the various signs taken as indicators of nervous system activation are not always in perfect agreement. Activation cannot therefore be a unitary variable, and care must be exercised to specify exactly what measures of nervous system activity are taken as indicators of activation in any particular instance. Third, pupillometric measures, although classically associated with more central indicators of brain activation (*13*), are still peripheral autonomic signs and quite removed from those central processes that one would wish to study directly. Nonetheless, the close relationship between peripheral patterns of pupillary dilation and the level of cognitive processing observed in the present experiments provides strong support of Jackson's idea that vigilance is a critical parameter of the higher information-processing functions of the human brain.

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