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NEUROPEPTIDES AND THEIR RECEPTORS: A PSYCHOSOMATIC NETWORK

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A major conceptual shift in neuroscience has been wrought by the realization that brain function is modulated by numerous chemicals in addition to classical neurotransmitters. Many of these informational substances are neuropeptides, originally studied in other contexts as hormones, "gut peptides," or growth factors. Their number presently exceeds 50 and most, if not all, alter behavior and mood states, although only endogenous analogs of psychoactive drugs like morphine, Valium, and phencyclidine have been well appreciated in this context. We now realize that their signal specificity resides in receptors (distinct classes of recognition molecules), rather than the close juxtaposition occurring at classical synapses. Rather precise brain distribution patterns for many neuropeptide receptors have been determined. A number of brain loci, many within emotion-mediating brain areas, are enriched with many types of neuropeptide receptors suggesting a convergence of information at these "nodes." Additionally, neuropeptide receptors occur on mobile cells of the immune system; monocytes can chemotax to numerous neuropeptides via processes shown by structure-activity analysis to be mediated by distinct receptors indistinguishable from those found in brain. Neuropeptides and their receptors thus join the brain, glands, and immune system in a network of communication between brain and body, probably representing the biochemical substrate of emotion.

The ongoing explosion in the recognized number of behavior-modifying chemicals present in brain has revolutionized the theoretical framework of neuroscience. Many newly identified neuromodulators are neuropeptides, i.e., short, signal peptides, which upon enzymatic cleavage from their polypeptide precursors, produce receptor-mediated behavioral effects. As we shall see, the term "neuropeptide" now includes many peptides described originally in other seemingly unrelated contexts as "hormones" (e.g., insulin) or "growth factors" (e.g., transferrin). We will describe the defining features of neuropeptides, emphasizing their pattern of receptor distribution throughout the brain and body in a network of potential information exchange among the brain, glands, and—as recent work from our laboratory demonstrates—the immune system.

A number of mood modifying drugs, including morphine (1), Valium (2), and phencyclidine (angel dust) (3, 4), have been shown to act by mimicking receptor interactions with endogenous neuropeptide ligands. Because a vast psychopharmacologic literature (5) rigorously documents that drugs which modify observable behavior simultaneously alter perception, memory storage, and mood, we can thus surmise that neuropeptides have a physiologic role in the regulation of behavior and emotional tone. Biochemical strategies for labeling brain receptors can reveal the specific neuroanatomical sites containing the target receptor molecules where behavioral alterations are initiated. Thus, brain/drug receptors now form an experimentally accessible link between the classical scientific system of knowledge spanning the disciplines of psychology, ethology, and neuroanatomy and the more recently evolved system of knowledge encompassing biochemistry, immunology, and molecular biology. Chemical neuroanatomy has become an exciting level of inquiry into nervous system function as it brings behavior and molecules into the same hierarchy of knowledge.

Strategy for validating brain receptor binding. Unfortunately, chemical purification of the receptors where drugs and endogenous brain chemicals initiate their effects on behavior has proceeded at a frustratingly slow pace. The strategy of examining a number of chemical analogs for their comparative ability to displace binding of radiolabeled receptor ligands and produce behavioral or other physiologic alterations has therefore proved an important strategy for providing a rigorous link between biochemistry and behavior. For example, numerous synthetic and semisynthetic opiate alkaloids and peptides have been shown to displace the binding of radiolabeled naloxone from brain membranes and brain slices prepared for autoradiography with the identical ($p < 0.001$) rank and absolute orders of potency displayed by these chemicals in raising analgesic thresholds in rodent behavioral tests (6, 7) and suppressing electrically induced contractions of the guinea pig ileum (8). In other words, "weak" opiates, e.g., those enantiomers of natural morphine with the "wrong" three-dimensional structure for binding to receptors, simultaneously require doses several orders of magnitude higher to elicit their pharmacologic effects and to inhibit receptor binding. All radioligand binding visualized on brain sections (Fig. 1) has been demonstrated to be displaced by the appropriate concentrations of numerous drugs and/or peptides.

Neuropeptide receptors, behavioral function, and classical neurocircuitry: focus on opiate receptor. Studies of opiate receptor distribution using this strategy have suggested that receptors throughout the neuroaxis are

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chemically identical (9, 10) but mediate different functions depending upon their neuroanatomical locus. Because performing well-controlled rodent behavioral experiments is much more time consuming than obtaining complete neuroanatomical maps of receptor distribution, only a very small percentage of the opiate (and other neuropeptide) receptors visualized can be confidently assigned to the mediation of specific behaviors. Comparison of the patterns of brain receptor distribution with patterns of radiolabeled neuronal projections have allowed us to surmise in one case that opiate receptors are associated with previously defined neuroanatomical circuitry (11). A network of neurons are strategically placed for communication among five distinct brain areas (olfactory bulb, amygdala, habenula, interpeduncular nucleus, and specific cellular groups in the lower brain stem reticular activating area). Behavioral experiments have proven that these areas are associated with olfaction, strong emotional memories, and overt sexual expression. For example, observations of mating behavior in rodents with lesions reveal that intact neurocircuitry in this five-link circuit is required for expression of normal mating behavior patterns. It is worth emphasizing that analogous neuroanatomical circuitry can be readily identified in the brains of all mammals. Thus, opiate-induced alterations in sexual behavior of laboratory rodents (12, 13) parallel those reported in the biologic psychiatry literature describing heroin and methadone opiate abusers.

Focus on insulin receptor. Recent studies of the distribution of validated ^{125}I-insulin binding to insulin receptors in rat brain have also revealed an association with classical neurocircuitry implicated, in this case, in the function of eating (14). Moreover, insulin receptors are clearly not uniformly distributed over brain capillaries, but also can be visualized in strikingly discrete patterns which coincide with classical layered fields of dendro- and axodendritic connections (e.g., substantia gelatinosa layer of the spinal cord) (14). Thus, although historically first known as a hormonal secretion of pancreas and a growth factor, insulin, which is indeed present in the brain (15), is a neuropeptide capable of modulating behavior (16).

Fundamental feature of neuropeptide receptors—enrichment at "nodal points": nodal points in the limbic system of brain. A fundamental feature shared by all neuropeptide receptors whose brain distribution has been well studied is profound enrichment at a number of the same brain areas. Many of these neuropeptide receptor-rich areas can be found within an intercommunicating conglomerate of brain structures classically termed "the limbic system," (17, 18), which is considered to mediate emotional behavior; in unanesthetized humans undergoing brain stimulation as a preclude to surgery for epilepsy, far-ranging emotional expression can be elicited by stimulation of cortex near the amygdala, the core of the limbic system. The amygdala, as well as the hypothalamus and other limbic system-associated structures, were found initially to be enriched in opiate receptors in monkey (19) and human brain (20). Later maps of numerous other neuropeptide receptors in brain [including substance P (21), bombesin (22), cholecystokinin (CCK) (23), neurotensin (24), insulin (14) and transferrin (25)] have continued to implicate the amygdala and other limbic system-associated structures (e.g., the cingulate cortex) as a source of receptor-rich sites where mood presumably is biochemically modified.

Some other nodal points in the CNS: dorsal horn of the spinal cord and the periaqueductal gray matter. A generalization first used to describe autoradiographic distribution of opiate receptors in rat brain (11), but equally applicable to that of most (if not all) neuropeptide receptors is enrichment at areas within the CNS where incoming, sensory information is processed. Thus, the dorsal horn of mammalian spinal cord (Fig. 1, a) where neurons transmitting information from glands, skin, and other peripheral organs make their first synaptic contact with the central nervous system, is enriched with virtually all neuropeptide receptors. Although it has not previously been considered part of the limbic system, neuropeptide receptors here, as postulated for other sensory way-stations (26), may filter and prioritize incoming sensory information so that the whole organism's perception is most compatible with survival.

Another neuropeptide receptor-rich locus in the CNS where peripheral information is integrated to modulate sensory thresholds is the periaqueductal gray region of the brain stem. This brain stem area has been well studied as a site where exogenous opiate analgesia is mediated (27). It is in direct synaptic communication with descending processes from the most recently evolved portion of mammalian brain, the frontal cortex (Fig. 1, c), and is thus thought to mediate the well-documented effect of expectation and conscious control on pain perception (28). Virtually every neuropeptide tested by microinjection through cannulae placed in this region has significant effects on pain thresholds. Neurotensin, bombesin (22), bradykinin, VIP (29), CCK (23), calcitonin (30), and substance P have all been shown to modulate pain thresholds in rodents (31), and receptors for these neuropeptides are found throughout this brain area, each in its own unique and reproducible pattern.

Nodal points in the body—outside the CNS. Neuropeptides and their receptors can be found throughout the body as well as brain. The entire gastrointestinal tract, for example is lined with networks of morphologically identifiable neuropeptide-containing cells and their receptors, which generally share similar specificity with receptors in brain. The human testis is as rich a source of messenger RNA for the opiate peptide proopiomelanocortin as the pituitary gland (32). A large body of interdisciplinary (endocrinology/physiologic psychology) literature demonstrates that, to cite one well-studied example, food appetite can be influenced by opiatergic modulation (33), partially by acting at pancreatic opiate receptors (34) in the periphery. A direct neuronal connection has been demonstrated between the pancreas and the nucleus ambiguous, a small brain stem structure rich in some neuropeptide receptors. However, it is now quite clear that classical synaptic "hookups" are not necessary for communication between distant sites within the organism, or even within the brain.

Numerous recent studies have shown that it is the rule rather than the exception that sites of neuropeptide storage in brain lack physical juxtaposition with their receptors; thus the classical, closely juxtaposed synapse between the neurotransmitter acetylcholine and its receptor on skeletal muscle is not at all typical of neuropeptides. Classical synaptic neurotransmission produces in-

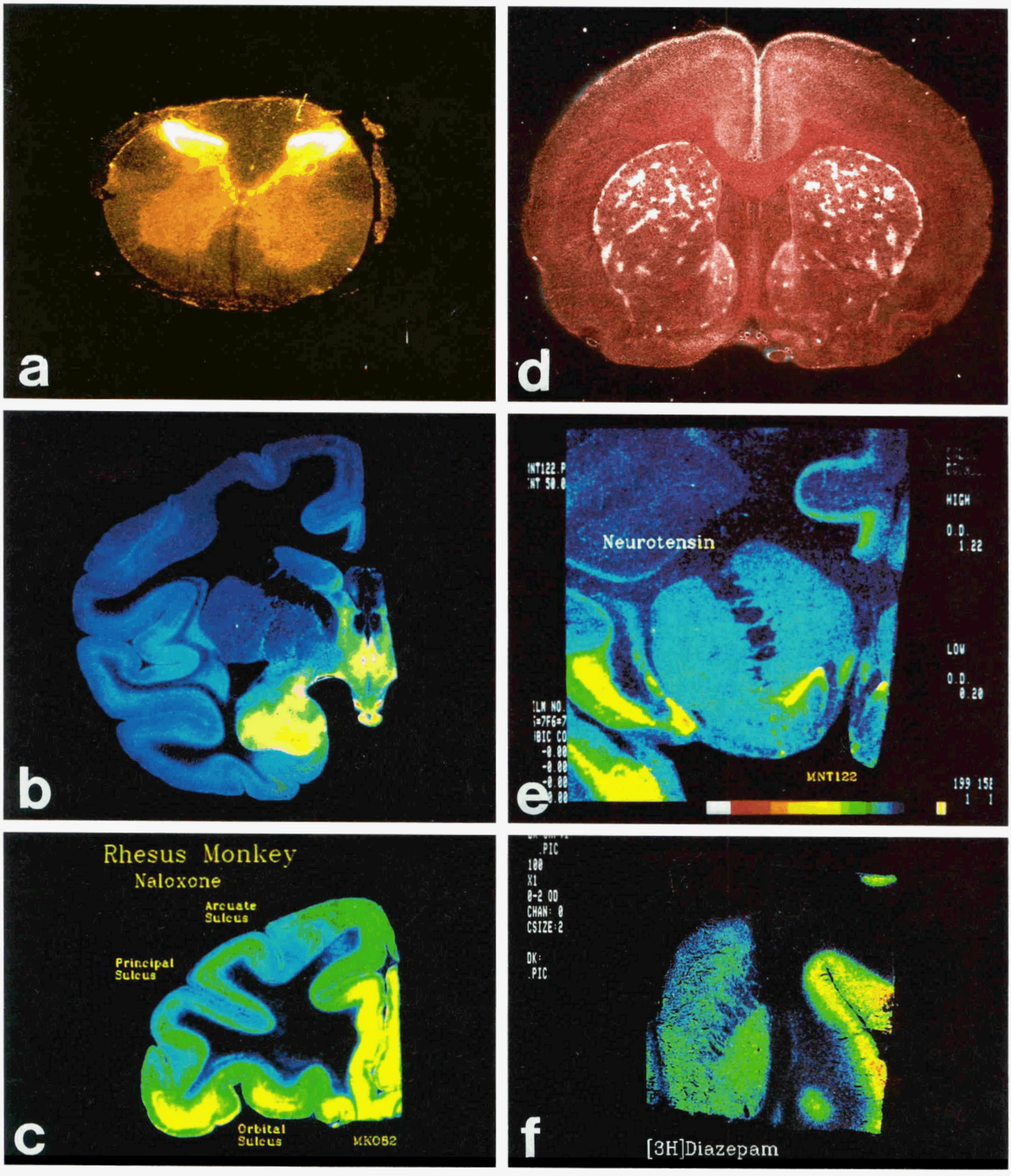

Figure 1. Autoradiographic localization of receptors (59) in the brains of rat (d) (60), rhesus monkey (a to c, e) (61, 62) and human (f) (63, 64). Cryostat-cut sections of fresh frozen brains were thaw-mounted onto slides, dried, and then incubated *in vitro* in solutions containing [^{3}H]-naloxone to mark opiate receptors (a to d), [^{3}H]neurotensin to mark neurotensin receptors (e), and [^{3}H]diazepam to mark benzodiazepine receptors (f). The specific distributions are visualized by darkfield microphotography of the sections coated with a tritium-sensitive emulsion, in which reduced silver grains show up collectively as whitish areas (a, d), or by computer-generated spectral coloring of densitometry data (59) digitized from tritium-sensitive film (b, c, e, f), in which reds and yellows code areas with dense receptors, green intermediate, and blue low density (color bar shown in e). Thematically, a to c show three "nodal" points in rhesus monkey brain. Opiate receptors are dense in the dorsal horn of the spinal cord (a), the hypothalamus and amygdala (b), and in frontal association and limbic cortex (c). These represent possible sites where neuropeptides can influence primary sensory (pain) inputs (a), motivational (limbic) processing (b), as well as higher cortical control of both (c). The remaining autoradiographs (d to f) show three different receptor systems in the corpus striatum of three different species. Specific distribution patterns can be correlated with other chemical and anatomical features in an effort to understand the unique role that the striatum plays in behavioral function.

formation transfer very rapidly on a time scale measured in milliseconds. The separate discipline of endocrinology has featured a general theoretical framework in which peptide hormones are synthesized, stored, and released from one organ while acting elsewhere, the clarity of communication residing in the specificity of receptors rather than physical juxtaposition. Neuropeptides probably share an analogous communication principle, with receptors serving as targets for circulating levels of neuropeptides, themselves produced at other loci in the brain and body. Thus, intercellular communication throughout networks of neuropeptide-rich nodes which extend from the brain to the endocrine and, as we shall see, the immune system may integrate the internal milieu of the whole organism.

Neuropeptides as the biochemicals of emotion. Charles Darwin (35) assumed that the physiologic basis of emotions—so invariant and identifiable in humans of all cultures as well as other primates—would one day be understood. The striking patterns of neuropeptide receptor distribution in mood-regulating areas of brain, as well as their role in mediating communication throughout the whole organism, makes neuropeptides the obvious candidates for the biochemical mediation of emotion. Does each neuropeptide bias information processing uniquely when occupying receptors at nodal points within the brain and body? If so, each unique neuropeptide's "tone" might produce a typical mood state. The opiate peptides clearly mediate a state of intensely reinforcing pleasure, while substance P release has been associated with pain (21) and indeed has a reciprocal relationship with opiatergic neurons at most levels of the neuroaxis (36). Microinjections of the neuropeptide angiotensin II through narrow cannulae implanted precisely in the angiotensin receptor-rich subfornical organ of rat brain (37) induces drinking behavior in seconds, while angiotensin receptors in the kidney with the identical structure-activity relationship of those in brain (37) apparently mediate retention of water from the kidney's collecting tubules. Thus, enhanced angiotensinergic tone appears associated with thirst and the conservation of water at several levels in the whole organism. More typically, neurotensin is a neuropeptide with very little known about its functional role; it has the typical neuropeptide receptor distribution in brain, similar receptors in kidney (38), and the ability to simulate histamine secretion from mast cells (39). Although experimental observations have been confined to a demonstration that neurotensin receptors on dopamine-secreting neurons modify some locomotory behavior in rats (40), we have no clue regarding the mood state induced by increased neurotensinergic tone at nodal points.

Neuropeptides in the immune system. We have utilized these examples from the nervous system to develop the concept that neuropeptides and their receptors form a network of information exchange which extends throughout the brain and body, including the immune system. For some neuropeptides, such as neurotensin, more information is available on their role in regulating mast cell histamine release than their function in the brain. Other neuropeptides have only recently been studied for their effects on immunologic parameters. An emerging literature indicating a role for opiate peptides in immune function (41) has encouraged us to consider the effects of other neuropeptide ligands which effect mood and behavior.

The benzodiazepines, including the drugs Librium and Valium widely prescribed for their anti-anxiety and sleep-inducing effects, act at receptor molecules in the brain and periphery (42) which normally serve as receptors for an endogenous anxiety-producing octadecaneuropeptide whose structure has very recently been sequenced (2). The evidence that the pharmacologically relevant receptors for radiolabeled diazepam (Valium) are indeed being mapped (Fig. 1, f) is provided by a correlation ($p < 0.001$) between the dose of a series of diazepam analogs in alleviating anxiety in humans and inhibiting binding to brain receptors. We have recently demonstrated specific benzodiazepine receptor binding on a B cell hybridoma with identical structure-activity displacement profiles previously documented on a number of other non-neuronal cells. In this case, the rigorous demonstration of stereospecific benzodiazepine receptor binding (Fig. 2) has come before the identification of a clear immunomodulatory effect, which has yet to be described. We have also shown that human monocytes, in a classical Boyden chamber assay, chemotax in response to low concentrations of benzodiazepines (Fig. 3; Reference 43) and that this response is completely blocked by the antagonist for this receptor, PK-11195.

Human monocytes will chemotax toward low concentrations of a number of neuropeptides, including opiates (44, 45), substance P (46), and bombesin (47). The receptors mediating chemotactic processes appear to be similar if not identical to previously characterized brain receptors, sharing appropriate structure-activity relationships in chemotactic potency as well as complete blockade of chemotactic affects by specific pharmacologic antagonists. For example, opiate receptor-mediated chemotaxis (Fig. 4) is triggered by opiate peptides but only weakly, if at all, by opiate alkaloids. Opiate peptide-induced chemotaxis is stereospecifically and completely blocked by the active opiate antagonist (−)-naloxone but unaffected by the same (10^{-8} M) concentration of its pharmacologically

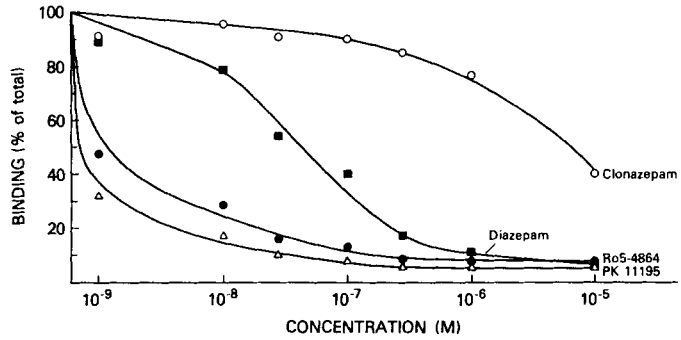

Figure 2. Displacement of [^{3}H]-Ro5-4864 binding to LK35.2 cells by unlabeled benzodiazepines. LK35.2 cells (65) were cultured in RPMI-1640 media supplemented with 10% fetal bovine serum, 1× nonessential amino acids, 1 mM sodium pyruvate, 2 mM L-glutamine and 5×10^{-5} β-mercaptoethanol. Cells were washed twice in 0.05 M Tris, 0.15 M NaCl, pH 7.4, and were suspended in a total volume of 0.5 ml of the same buffer. Displacement potencies of Ro5-4864, PK-11195, diazepam, and clonazepam were determined by incubating cells with 2 nM [^{3}H]Ro5-4864 in the presence or absence of various concentrations of cold ligands ranging from 10^{-5} to 10^{-9} M. After a 30-min incubation at 4°C the cells were assayed for [^{3}H]Ro5-4864 binding by rapid vacuum filtration over glass fiber filters (Whatman GF/C) followed by washing twice with 4 ml of cold (4°C) buffer. The displacement curves shown are the total percentage of triplicate determinations of [^{3}H]Ro5-4864 binding occurring in the presence of each drug over the indicated concentration ranges.

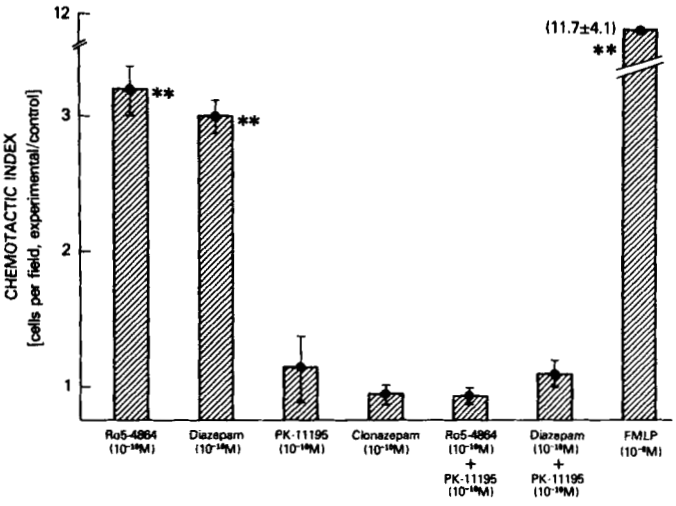

Figure 3. Benzodiazepine receptor-mediated chemotaxis of human monocytes (43). Chemotaxis assays were performed by using modified Boyden chambers as described (43, 45) in which the upper and lower compartments were separated by 5-μM pore sized polycarbonate filters. Migration was assessed after a 90-min incubation at 37°C. Cells were enumerated after fixing and staining using an image analyzer by counting three fields in triplicate. Data are expressed as a migration index. The number of migrating cells in the buffer alone controls was 40 cells/field (200×). Values shown represent the mean and SEM of four experiments. Ro5-4864, (4-chlorodiazepam), and diazepam yielded statistically significant responses compared to control (**, $p < 0.001$). Clonazepam, a benzodiazepine analog with low activity on non-neural cells, was not active in this assay. These receptor-mediated events were blocked by the appropriate antagonist, PK-11195, when co-mixed with the agonist Ro5-4864 or diazepam. The response of the tripeptide, f-Met-Leu-Phe (FMLP) was not inhibited when co-mixed with equimolar (10^{-8} M) PK-11195.

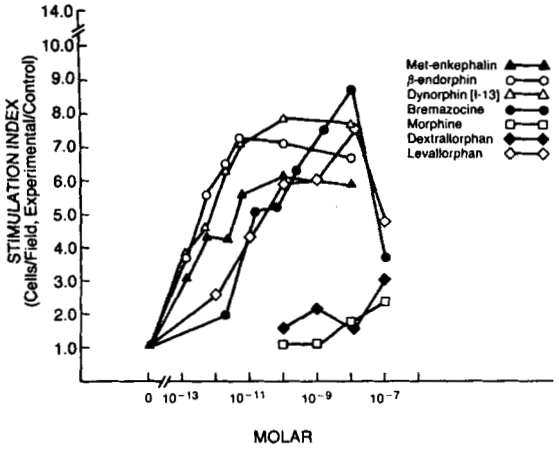

Figure 4. Chemotactic response of human monocytes to opiates and opiate peptides (45). Chemotaxis was performed as described in Figure 2. Data is expressed as a stimulation index. The maximal response for all analogs occurred at 10^{-8} M, although the peptides β-endorphin, D-Ala-D-Leu enkephalin, and dynorphin [1-13] were similarly active at 10^{-10} M. The EC_{50} for these agents was 5×10^{-12} M. These responses were stereospecifically reversed by naloxone (45). Stereospecificity is demonstrated in this figure by levallorphan, which shows intermediate activity compared to opiate peptides, while its enantiomer, dextrallorphan, is inert. The stimulation index for the chemotactic peptide f-Met-Leu-Phe, 10^{-7} M, was 14.2.

inert optical isomer (45).

The existence of distinct, discrete neuropeptide receptors on human monocytes is further suggested by the finding that chemotaxis can only be blocked or antagonized with the appropriate selective antagonist for a given neuropeptide receptor class. For example, chemotaxis via the f-Met-Leu-Phe receptor, while antagonized by specific f-Met-Leu-Phe antagonists, is not affected by the opiate (45) or benzodiazepine (43) receptor antagonists naloxone or PK-11195, respectively. The physiologic significance of this elegant *in vitro* model system is yet to be convincingly identified; however, the existence of neuropeptide receptors on monocytes, known to interface with numerous components of the immune system and body, suggests an exciting potential substrate for mediating interactions between the brain and immune system.

The penetration of immune and endocrine systems by cells and products of the nervous system appears to be mutual. Thus, cells and products of the immune system exist in close physical, as well as communicative, contact with the nervous and endocrine systems (see E. Blaloch and D. Felton, this volume). Hemopoietic stem cells have been identified in the CNS (48), and the brain, only 10% of whose cells are neurons, is extensively populated by cells of macrophage derivation, the microglia. Other glial cells produce, *in situ*, hormones such as IL-1 (49, 50) which have been largely studied as immunologic factors. IL-1 has profound effects on CNS function, including thermoregulation and sleep induction, and therefore may have receptor sites within the CNS where it may function as a neuropeptide. Immune hormones, like the interleukins or interferons, may be precursors for peptides which act within the brain to also alter behavior. Macrophages are capable of transition from one body compartment to another and as such could serve as a kind of "mobile synapse," conveying information from one body compartment to another through a physical translocation, a concept commonly applied to intra-immune system communication (51).

Conclusion. Clearly, the conceptual division between the sciences of immunology, endocrinology, and psychology/neuroscience is a historical artifact; the existence of a communicating network of neuropeptides and their receptors provide a link among the body's cellular defense and repair mechanisms, glands, and brain. Roth and colleagues (52) have emphasized that neuropeptides and their receptors are highly conserved in evolution. Opiate peptides (53) and insulin (54) have been identified in unicellular organisms. Thus, neuropeptides have been a stable feature mediating intercellular communication throughout evolution. In higher animals they have unique neuroanatomical distributions which allow us to conceive of them as biochemical mediators of the emotions. Assuming that their function as well as their structure is conserved in evolution, then even the most primitive organisms must utilize neuropeptides to bias behaviors toward those with the greatest survival value. The functional integration of the body's cells (55) through networks of neuropeptides and their receptors (56) would be expected to be critical to the health of the organism as a whole. An older psychologic literature (57), recently beginning to receive more attention (58), suggests that emotional states can significantly alter the course and outcome of biologic illnesses previously considered to be strictly in the somatic realm.

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Note Added in Proof: F. O. Schmitt has thoroughly and elegantly summarized (66) the recent conceptual shift that has occurred in neuroscience due to the expansion

of recognized neuromodulators or informational substances," particularly the neuropeptides. This important theoretical work contains some similar viewpoints on the evolutionary stability of neuropeptides, and "parasynaptic" modes of communication between noncontiguous neurons made possible by receptor specificity.

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