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A concise review of concepts in opioid pharmacology up to the discovery of endogenous opioids

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Abstract:

This brief review covers concepts in opioid pharmacology that were promoted during the period leading up to the establishment of the International Narcotics Research Conference (INRC) in the early 1970s and the discovery of endogenous opioid peptides in 1975. The founders of INRC, meeting together during the International Union of Pharmacology meeting in Basel in 1969, recognized that the time was ripe for the creation of an international society that would provide a venue for the discussion of research across disciplines in this rapidly expanding area of science. The emphasis here is on studies leading to the demonstration that specific receptors for morphine-like analgesics exist, the search for endogenous ligands for these receptors, and early attempts to elucidate the mechanisms underlying opiate drug tolerance, dependence and addiction.

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Significance Statement:

Research on opioids in the 20th century was driven by the search for non-addicting analgesics. This review discusses the development of the "analgesic" receptor concept, the demonstration that such receptors existed, and the search for an endogenous ligand. Conceptual models were proposed to explain tolerance to the actions of opiate drugs and the development of dependence and addiction. This review explains these models and indicates how they foreshadowed more recent discoveries on the acute and chronic actions of opiate drugs.

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Research on opioids expanded substantially in the first half of the 20th century, triggered in the USA by concerns linked to the increased awareness of opiate drug addiction, leading to the passage of the Harrison Narcotics Act of 1914 and the increasing availability of Federal government funding for this research. Half a century later these trends, together with comparable expanding interest and opportunities outside the USA, set the stage for major breakthroughs in opioid research. The fundamental concept that dramatically expanded interest in research on opioids in the middle of the century was the growing understanding during the 1950s and 60s that mammals expressed specific receptors that were activated by morphine and related non-endogenous compounds, agents with which most individuals would never naturally interact. In this short essay, I will discuss the development of the opioid receptor concept and its implications leading to the search for endogenous opioids, and the parallel development of ideas seeking to understand opioid tolerance and dependence, all initially driven by the search for non-addicting analgesics. Since space (and reader's time) is limited, the choice of papers to consider is inevitably selective and personal, with emphasis being placed on the papers that had most impact on developing concepts related to opiate drug action.

How does morphine exert its analgesic action?

Semi-synthetic analogs of morphine such as heroin, and new drugs with similar pharmacology such as methadone and meperidine (pethidine), were first developed early in the 20th century, and were all initially marketed as non-addicting analgesics, unlike morphine or raw opium (see review by Bretcher, 1972). By the 1920s it was clear that claims that these drugs were not addictive could not be substantiated. (The much more recent introduction of oxycodone as a "safe" analgesic with low abuse potential bears a frighteningly similar resemblance to this earlier history). The search for non-addicting opiate analgesics began, generally driven by medicinal chemists who adopted two alternative strategies; systematic modification of the structure of morphine itself to alter its pharmacology, or searching for alternative novel chemical structures that might be potent analgesics without addictive properties. Studies were conducted in laboratory animals, predominantly rats and mice, but dogs and other domestic species were also

used more extensively than now. Initial comparisons of drug actions were very descriptive, but the need for quantitative comparisons required that appropriate tests for analgesic activity were developed. Mechanical pain and heat were often used as transient acute nociceptive stimuli, as in the case of the tail-flick test developed by D'Amour & Smith (1941), and the hot-plate test (Eddy & Leimbach, 1953) in rodents, or mechanical stimuli applied to the skin of dogs (Gilbert & Martin, 1976). Morphine and related drugs increase reaction time in these tests, but other drugs (for example, aspirin, acetaminophen) are essentially inactive. In retrospect, it is therefore not surprising that active novel chemical agents discovered with these assays shared many pharmacologic properties with morphine and other opiates.

However, by the 1950s, it was apparent that modifications of the morphine structure could substantially alter its pharmacologic properties. Arnold Beckett (1952), a medicinal chemist at Chelsea College in London, summarized the work of his group and several others, identifying critical structural features necessary for analgesic action in compounds related to morphine and other chemical series (including compounds related to meperidine and methadone). Beckett suggested that “the minimum requirement for activity may be a “hydrophobic group……containing a basic centre with an overall optimum spatial arrangement”, and went on to emphasize the significance of stereochemistry since he and others had shown that in enantiomeric drug pairs, one enantiomer was much more potent as an analgesic than the other. On this evidence, Beckett stated that for analgesic action. “the stereochemical configuration of the drug must be complementary to that of a certain tissue surface or enzyme system”. He did not in this paper use the term “receptor” but two years later Beckett & Casy (1954) specifically proposed the existence of a receptor for morphine, presenting additional data on the stereochemistry of morphine-like analgesics, emphasizing that the active enantiomers in several chemical series showing analgesic activity all had the same absolute stereochemical structure (relative to D-alanine), similar to the configuration of natural morphine, the D (-)-isomer (levorotatory) of a structure that has several centers of asymmetry.

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Beckett & Casy (1954) also noted the critical role of the substituent on the basic nitrogen atom. Increasing the size of this substituent tended to reduce analgesic activity. There was much interest in the properties of the N-allyl substituent; nalorphine (N-allynormorphine) was known to antagonize the analgesic and respiratory depressant actions of morphine, as well as other morphinans, methadone, meperidine and other drugs with similar properties. Replacement of the N-methyl of morphine with N-propyl or N-isobutyl also yielded compounds showing a gradation of analgesic and anti-analgesic actions, emphasizing the significance of the structures around the critical basic nitrogen in ensuring an appropriate fit to a postulated receptor surface, and this work was soon extended to the structural correlates of analgesic and anti-analgesic actions of other morphinan structures (Archer et al, 1962).

On the basis of the stereochemical specificity required for opioid activity in several chemical series, and the ability of relatively small changes in drug structure around the nitrogen atom to change activity from analgesic to antagonist, Beckett & Casy (1954) proposed that opiate drugs acted through specific receptor and they presented a cartoon of a hypothetical receptor surface. (Fig 1A). Beckett and Casy's pioneering studies on receptors for morphine and related analgesics were hampered by the quantitative limitations of the *in vivo* assays then used to measure analgesic activity, and the difficulties of comparing quantitative measures of potency across species (in particular between studies of experimental pain in rodents and effectiveness in the relief of pain in man when very different measures were used in each species. Until the 1950s, the only accepted methods of quantifying opioid potency required measurement of responses to noxious stimuli such as heat or pressure in laboratory animals relative to verbal reports of pain intensity in human subjects. A few studies of opioid actions on the gastrointestinal tract and inhibition of the peristaltic reflex, both *in situ* and in isolated gut segments, had been conducted early in the history of pharmacology (e.g., Trendelenburg 1917; Schaumann et al, 1952) although there was no consensus on whether the actions of opiates in the intestine were mediated by mechanisms similar to those involved in pain relief. In the 1950s, work on opiate drug effects on peripheral tissues expanded significantly, utilizing the greatly expanded range of opiate and related

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drugs that had by then become available and a wider variety of peripheral tissue preparations. Kosterlitz and colleagues in Aberdeen, in particular, published a series of studies evaluating opioid actions on *in vitro* preparations of various peripheral tissues innervated by the autonomic nervous system (Kosterlitz & Robinson 1957; Gyang et al, 1964), and Paton at Oxford published a widely cited paper (Paton, 1957) demonstrating that low concentrations of morphine and other opiates could reduce the release of acetylcholine from the myenteric plexus of isolated guinea pig ileum segments (Fig 2). On the basis of this study, he proposed that evaluating the effects of drugs on the release of neurotransmitters from the intestinal myenteric plexus offered an accessible way to evaluate potential drug effects on transmitter release in the brain - the myenteric plexus was a miniature paradigm of the central nervous system (Paton, 1957).

There was also increased emphasis on quantitative analyses of opiate drug actions in these peripheral preparations. Since Schild and colleagues had shown that receptors could be defined by their sensitivity to antagonism by comparing the apparent affinity constants for receptor selective antagonists at receptors in different tissues and across species (Schild 1957; Arunkulana and Schild, 1959), quantitative measures of antagonism of morphine actions by nalorphine, and later naloxone and other antagonists in isolated tissue preparations were compared with similar estimates of antagonism of the analgesic or other actions of morphine and other opiate drugs *in vivo* (Gyang & Kosterlitz, 1964; Cox & Weinstock, 1964; 1966). These results were complicated by the partial agonist properties of nalorphine and, as discovered later, its affinity for both □ and □ opioid receptors. Nevertheless, these results suggested that the receptors mediating opioid action in peripheral tissues were quantitatively similar in terms of sensitivity to nalorphine to the receptors in the central nervous system that were activated by morphine to induce analgesia. The discovery that naloxone was a pure antagonist of the actions of morphine and other opiates (Kosterlitz & Watt, 1968; McGilliard & Takemori, 1978), both with respect to a inhibition of acetylcholine release in the gastrointestinal tract and to analgesia, in contrast to the more complex actions of nalorphine, led Kosterlitz to propose that antagonism of a pharmacologic effect by

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naloxone should be considered a critical criterion for a role for opiate receptors in the mediation of this action.

Despite the demonstration of the similarity of the receptors for morphine in the brain and in peripheral tissues, the extension of structure-activity studies to an increasingly wide range of chemical structures and improvements in assay methodology began to expose anomalous results as more quantitative comparisons of analgesic and antagonist activities were obtained. An important study by Portoghese (1965) noted that there were at least two conformations of the phenylpiperidine moiety in different chemical series capable of exerting potent analgesic activity, and that the ability of nalorphine to antagonize some potent analgesics was reduced relative to its ability to antagonize morphine. On the basis of these differences and a detailed analysis of the analgesic potencies of several chemical series, he proposed, with remarkable foresight, that there were either differences in the way different chemical entities interacted with the proposed “analgesic” receptor (Fig 1B), or that there might in fact be more than one receptor mediating the analgesic actions of different opiates. Many years later, both of these predictions have been confirmed. The suggestion that there may be more than one receptor responsible for the actions of opiate drugs was soon supported by Martin and colleagues (Gilbert & Martin, 1976) studying the actions of drugs related to cyclazocine in dogs. They suggested the existence of three receptor types mediating the actions of morphine and related drugs: the μ receptor responding preferentially to morphine, the κ receptor responding preferentially to ethylketocyclazocine, and the σ receptor responding predominantly to SKF 10,047 (Gilbert & Martin, 1976). Further support for the existence of more than one type of receptor for morphine like drugs soon emerged from detailed studies of the actions of endogenous opioid peptides (Lord et al 1977). The existence of μ and k receptors, the products of different genes, has now been unambiguously established. Two additional opioid receptor types, the δ- receptor identified by Kosterlitz et al (1976) and the nociceptin/orphanin FQ receptor (also known as the ORL-1 receptor; Mollereau et al, 1994) were later described as additional members of the opioid receptor family. The σ receptors initially proposed by Gilbert & Martin (1976) also exist but are now known to be structurally very different from the

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family of opioid receptors (Maurice & Su, 2009). With access to a greatly expanded range of compounds and to additional more quantitatively reliable measures of receptor activity more closely related to the immediate consequences of receptors activation (agents and techniques that were not available to Portoghese or to Martin and colleagues), Portoghese's alternative 1965 proposal, that different opioid drugs may interact with the same receptor through different orientations and modes of interaction, has been supported recently in studies of agonist "bias" towards alternative down-stream signaling pathways as a result of preferential interactions of different agonists with different receptor conformations (Schmid et al, 2017).

The conclusions of Beckett, Portoghese, and others that the remarkable analgesic properties of morphine and related drugs required activation of specific "analgesic" receptors encouraged several research groups to attempt to purify these receptors from neural tissues. Several studies were directed at locating the precise target sites in the nervous system to which opiate drugs required access in order to induce an analgesic response, using either discretely localized injections of drug into brain (Herz et al, 1970; Dey & Feldberg 1976) or spinal cord (Yaksh & Rudy, 1976), or by evaluating the distribution in brain of radiolabeled opiate after injection into the cerebral ventricles (Ingoglia & Dole, 1970). These studies, and others, collectively pointed to the importance of actions in the periaqueductal grey region, the rostro-ventral medulla, and the dorsal horn of spinal cord.

Isolation of the proposed morphine receptors was more challenging. Since the proposed receptors could be activated or inhibited by low concentrations of specific agonists or inhibitors, binding of a highly potent drug to a receptor molecule appeared to present a viable approach to receptor isolation. It was also recognized that the likely concentration of the analgesic receptors in brain would be very low, relative to the high concentrations of structural proteins and lipids; the number of specific binding sites would be very low relative to the very high number of non-specific binding sites. Goldstein et al (1971) proposed a solution for this problem, taking advantage of earlier studies demonstrating the stereoselectivity of the receptors for morphine-like

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analgesics. Specific binding sites should be stereoselective, high- affinity and saturable, while non-specific binding sites should be neither stereoselective nor saturable. Specific binding could be quantified by measuring the binding to tissues of a radiolabeled highly-potent analgesic drug and computing the difference in binding of the radioligand in the absence and presence of a receptor-saturating concentration of an unlabeled competitor drug. This insight proved critical to the eventual demonstration of specific ligand binding to opioid receptors. Selection of the appropriate tissue preparation in which to detect specific binding also presented problems. Brain tissue was the obvious choice, but because of the anticipated low concentration of specific binding sites, it was assumed that some fractionation of brain tissue to generate fractions enriched in opioid receptors would be needed. The Goldstein group elected to use a lipid fractionation technique, showing that a stereospecific fraction of radiolabeled levorphanol binding in mouse brain homogenate could be extracted into chloroform: methanol (Goldstein et al, 1971; Lowney et al, 1974). Later, Horace Loh and colleagues demonstrated that liposomes from brain tissue that contained cerebroside sulfates also bound radiolabeled opiate drugs stereospecifically (Loh et al, 1974). However, the fraction of drug binding that was stereospecific in these studies was extremely small, and further attempts at purification did not yield products that had the anticipated properties or brain distribution of analgesic receptors. It is possible that the low levels of specific binding in these semi-purified lipid fractions was associated with low levels of contamination with receptor protein.

The breakthrough came in 1973. The research groups of Solomon Snyder in Baltimore, Eric Simon in New York and Lars Terenius in Sweden independently elected to study the binding of radiolabeled opiate drugs to membrane suspensions isolated from brain homogenates from rats or mice. Two technical developments were critical to the detection of the very low levels of specific binding to the receptors in the face of a high level of non-specific binding; the first was the use of very high specific activity tritium-labeled opiate drugs which had only recently become available, making it possible to use for the first time very low (sub-micromolar) concentrations of the labeled opiate and thus reducing the non-specific binding to a markedly smaller fraction of the total binding

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of the radiolabel. The second development permitted a greater reduction of non-specific binding of the hydrophobic opiate drug molecules relative to the much smaller quantity of high affinity binding. This was addressed by using membrane “washing” techniques with cold buffer; by filtration over glass fiber filters (Pert & Snyder, 1973; Simon et al, 1973) or by centrifugation and re-suspension (Terenius, 1973). In this way, the low-affinity non-specific binding was markedly reduced while leaving the slowly dissociating high-affinity binding to the receptors still detectable in the filtered or pelleted membrane fraction. With these technical improvements, the Snyder, Simon and Terenius groups each were able to demonstrate unambiguously the existence of high affinity stereospecific binding sites with relative affinities for opiate drugs of several different chemical series that correlated closely with their relative analgesic potencies. These results were reported at the 1973 INRC meeting; it was almost 20 years before the genes coding for opioid receptors were finally identified (Evans et al, 1992; Kieffer et al, 1992).

The search for endogenous ligands for the “analgesic” receptor:

The demonstration by Pert & Snyder (1973), Simon et al (1973), and Terenius (1973) of high affinity stereospecific opioid binding sites in brain membranes confirmed the existence of specific receptors for morphine. This immediately posed the question – what is the normal function of these receptors? Harry Collier, one of the founders of INRC, formally proposed the existence of endogenous ligands for opiate receptors during the World Congress of Pharmacology in 1972 (Collier, 1972) and several labs began to seek endogenous ligands for the proposed receptors. One approach made the reasonable assumption that any endogenous ligand was likely to share a chemical structure with significant resemblance to that of morphine itself. The biochemical pathway to the synthesis of morphine in the poppy had recently been characterized, with the observation that tyrosine was a critical precursor in the synthesis (Kirby, 1967), making it plausible that a similar pathway might exist in mammalian brain. However, critical enzymes in the synthetic pathway in the poppy were not present in animals, so the existence of endogenous morphine, *per se*, seemed improbable. Around this time, it became possible to raise antisera directed against small molecules such as morphine.

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Investigators in the Goldstein lab and elsewhere screened brain extracts for materials binding to anti-morphine antisera, assessing the bound materials in opiate drug-sensitive bioassay preparations where antagonism by naloxone or nalorphine might be demonstrated, but results were not encouraging.

An alternative approach to identifying endogenous ligands for the receptors was to screen brain extracts for activity directly in the opioid bioassays, and this had the advantage that any observed activity could be evaluated directly by testing for antagonism by naloxone. This approach was successfully employed by John Hughes and Hans Kosterlitz in Aberdeen, who reported initial success, with no experimental details, in 1974, following this with a report of the identification by bioassay of opioid activity in extracts of porcine brain at the 1975 INRC meetings and a brief publication (Hughes, 1975). The bioassay preparation used by Hughes to detect opioid activity was the isolated mouse vas deferens preparation, previously described by Henderson et al (1972). In this preparation, the ability of naloxone to reverse or prevent the inhibitory effect of the porcine brain extract on smooth muscle contraction in response to nerve stimulation was clearly apparent. Later in the same year they published the definitive report of their discovery, isolation and characterization from porcine brain extracts of what turned out to be two closely related pentapeptides which they named Met- and Leu-enkephalin (Hughes et al, 1975). Both peptides showed typical opioid-like activity in two isolated tissue bioassays, the mouse vas-deferens assay and the guinea pig ileum assays previously used by the Kosterlitz group for structure-activity studies with opiate drugs. Antagonism of the actions of the synthetic peptides by naloxone was clearly observed. In this study, characterization of the amino acid sequence of the two peptides was achieved by mass-spectrometric analysis, one of the earliest applications of this technique for determination of the sequences of novel bioactive peptides.

Inhibition of the binding of radiolabeled opioid ligands to brain membranes expressing the opioid receptor was also a feasible way to identify potential ligands. The challenge in using this technique was similar to the challenges in the initial identification of the specific binding opioid binding sites; the need to discriminate specific inhibition of

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specific binding at the receptor with non-specific inhibition of binding unrelated to occupation of the active site. The Terenius and Snyder groups were able to demonstrate selective inhibition of specific opioid binding by partially extracts of brain tissues (Terenius & Wahlstrom, 1974; Pasternak et al, 1975). Later work showed that the materials isolated by the Terenius and Snyder groups was identical to the enkephalins previously isolated by Hughes, Kosterlitz and colleagues.

The Goldstein group had during this period been evaluating the possible presence of endogenous opioids in extracts of pituitary gland obtained either from a local slaughter house or in commercial extracts of the gland. Numerous bioactive peptides had been identified during the 1960s and shown to play major roles in the nervous and endocrine systems. Goldstein and colleagues therefore considered that an endogenous opioid might be a peptide even before the characterization of the enkephalins. Since the minimal structural requirements for activation of the morphine receptor had been defined by the chemists, Avram Goldstein attempted to design a small peptide that could interact with the previously defined features of the morphine receptor; a peptide with modest but significant opioid activity in the guinea pig ileum bioassay was eventually reported in 1975 (Goldstein et al, 1975), confirming the concept that a peptide could activate this receptor. A large number of endocrine related peptides had recently been discovered in pituitary gland. Using the isolated guinea pig ileum preparation as a bioassay, Goldstein and colleagues showed the presence of opioid agonist activity in the guinea pig ileum bioassay in extracts of bovine and porcine pituitary gland and confirmed that the activity could be antagonized by naloxone. Careful inspection of the properties of the different pituitary extracts suggested the presence of more than one active principle (Teschemacher et al, 1975; Cox et al, 1975a). Degradation of opioid activity in pituitary extracts by peptide-degrading enzymes (trypsin, chymotrypsin) indicated that the active principles were peptidic in nature.

Once the enkephalin structures were published in 1975, it was immediately noticed that the Met-enkephalin sequence was identical to the first five amino acids of the C-terminal

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fragment, β-lipotropin-(61-91), of the previously isolated pituitary peptide, β-lipotropin, a fragment that had been isolated from the pituitary gland and reported by two groups (Li, 1964; Bradbury et al, 1976). The opioid activity of β-lipotropin-(61-91) was quickly confirmed (Cox et al, 1975b: Bradbury et al, 1976; Feldberg & Smyth, 1976), and shown to have properties very similar to the material isolated from bovine pituitary by Teschemacher et al (1975). This peptide soon became known as β-endorphin. It was initially thought that β-endorphin was the immediate precursor of Met-enkephalin, but later work showed that there is no appropriate peptide cleavage site following the terminal methionine of met-enkephalin so that peptide processing enzymes in secretory vesicles cannot liberate Met-enkephalin from β-endorphin. Met- and Leu-enkephalin are both formed from a different precursor peptide, the product of the pro-enkephalin gene. The processing of larger peptide precursors to yield bioactive opioid peptides is discussed in detail in a companion paper in this journal (Fricker, 2020). Another highly potent opioid component of pituitary extracts, described initially by Cox et al (1975a), was later isolated and characterized, proving to be a novel highly basic peptide that was given the name, dynorphin, because of its very high affinity for its receptor (Goldstein et al, 1979; 1981).

Over the next decade the precursors for the three families of opioid peptides were identified, their genes cloned, and studies of their functions progressed. We now know that there are dozens of endogenous peptides with opioid activity; the properties of these peptides in reviewed in detail elsewhere in this issue (Fricker, 2020). Much of this work was first reported at INRC meetings, and many of the techniques and approaches first developed to study endogenous opioids were later employed widely in the study of other peptides isolated from the nervous system, thus facilitating the development of neuroscience in general.

Understanding opioid tolerance, dependence and addiction:
Experimental studies of the effects of morphine and its chronic administration appeared early, as the science of pharmacology was emerging from the earlier discipline of *materia medica*. Claude Bernard is credited by Tatum et al 1929 with a description of

the effects of morphine in frogs in 1864. The paper by Tatum, Seevers and Collins (1929) was itself an important summary of the acute and chronic actions of morphine and other opiate drugs in dogs, rabbits, cats, and monkeys that concluded with a proposed explanation of the symptoms that were observed when morphine treatment was stopped after a period of chronic administration. They noticed that in dogs low doses of morphine produced sedation with depression of respiration, but higher doses frequently caused seizures, with death often following closely after the seizures. Seizures and other signs of stimulation were also observed after high doses of morphine in rabbits, cats and monkeys. With chronic dosing, the sedative responses declined over time (i.e., tolerance was observed) but there was little tolerance to the convulsant effects of morphine; the lethal dose of morphine was not very different between naïve dogs and dogs pre-treated with a chronic morphine. To explain these results, Tatum et al (1929) proposed that during chronic treatment with opiates, tolerance developed to the sedative and depressant actions of the drug while there was no tolerance, and possibly sensitization, to the stimulant actions. When drug treatment was terminated, the residual depressant effects quickly decayed leaving the stimulant actions unopposed, resulting in what we now call the withdrawal or abstinence syndrome. Although the morphine withdrawal syndrome does not in fact closely resemble the stimulant effects of very large doses acute doses of morphine – morphine withdrawal is not usually accompanied by seizures, for example – this hypothesis was generally accepted over several decades as a plausible partial explanation of opioid tolerance and dependence. A critique outlining the major defects in this model was eventually published by one of the original authors, Maurice Seevers in 1962 (Seevers & Deneau, 1962).

The report of morphine actions in dogs and other species by Tatum et al (1929) and similar reports by other authors were essentially non-quantitative descriptions of the effects observed. Himmelsbach and colleagues realized the importance of quantitative studies, developing a quantitative model for evaluation of addiction, tolerance and abstinence in the rat. By 1938, Himmelsbach had moved to the US Public Health Service Hospital at the Federal Prison in Lexington Kentucky and started conducting

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studies of tolerance, withdrawal and addition in human subjects. Kolb & Himmelsbach (1938) and Himmelsbach (1939) published a method for the quantitative evaluation of the opioid abstinence syndrome in man using a scale that assigned points for each withdrawal symptom, including yawning, lacrimation, rhinorrhea, perspiration, mydriasis, tremor, gooseflesh, anorexia, restlessness, emesis, fever, hyperpnoea, rise in blood pressure and weight loss. The studies of Himmelsbach and other using this approach are among the first applications of quantitative methodology to the study of neuropharmacological actions of drugs in man. In a series of papers Himmelsbach and colleagues were able to provide a detailed time course of the opioid withdrawal syndrome, and delineate temporal differences in the withdrawal syndromes following chronic treatments with different opiate drugs, showing that the time of peak symptom severity was related to the rate of elimination of each drug. They also demonstrated that the severity of the withdrawal syndrome was dose-dependently related to the maintenance doses of drugs taken by the subjects prior to withdrawal (Kolb & Himmelsbach, 1938; Andrews & Himmelsbach 1944). These and similar studies with other addictive drugs were very important in defining differences between different pharmacologic classes in the nature of their withdrawal syndromes. It should be noted that although these studies were considered ethically acceptable at the time, the choice of subjects (prisoners) and the procedures to which they were subjected (presenting significant additional risks to the subjects) are now considered unethical and inconsistent with the principles for the ethical conduct of research in human subjects as defined in the Belmont Report (1979). Prisoners in the Lexington Federal Prison who volunteered for these studies in return for privileges within the prison and other inducements were subject to unacceptable coercion and risks that could not be approved by any modern Institutional Review Board.

Other groups also made important contributions to the quantitative assessment of opiate drug-induced tolerance and dependence in studies using laboratory animals. Goldstein & Sheehan (1969) demonstrated that the increase locomotor activity in mice induced by analgesic doses of morphine, which they called the "running fit" could be reliably quantified in different groups of mice. Chronic treatment with morphine or other

analgesics induced a marked tolerance to this action, thus providing an easily quantified measure of tolerance. In the same year, Way and colleagues (Way et al, 1969) quantified the escape jumping of chronically morphine-treated mice when given naloxone to precipitate withdrawal, providing a widely used measurement of withdrawal intensity.

By the mid-1960s the characteristics of tolerance and dependence on opiate drugs were becoming well established but the mechanisms underlying these actions remained mysterious. Several groups carefully evaluated the effects of chronic treatment with opiates on their metabolism, but unlike the barbiturates, chronic morphine treatment did not significantly increase (or decrease) the rate of its own metabolism – self-induction of hepatic drug metabolizing enzymes does not occur with morphine or most opiate drugs. Thus, metabolic alterations could not account for the high degree of tolerance that can be induced by chronic morphine dose regimens. This pointed to changes in the systems that were directly targeted by opiate drugs and ion down-stream signaling pathways. One of the first mechanistic explanations of tolerance and dependence, proposed independently by Shuster (1961) and Goldstein & Goldstein (1961; 1968) took advantage of then recent developments in the field of enzyme regulation, applying the concepts of enzyme repression and induction as mechanisms underlying the adaptive changes in neural function during the development of tolerance and physical dependence on opiate drugs. The regulation of enzyme synthesis was a hot topic in the world of biochemistry in the 1960s, following the pioneering work of Jacob & Monod (1961) in demonstrating that the rate of synthesis of many enzymes was directly controlled by repressors whose activity was regulated by the concentration of the products formed by the enzymes, thus permitting a “feed-back” control of enzyme synthesis based on the local concentration of enzyme product. The Shuster and Goldstein models were essentially direct applications of the basic principles developed by Jacob & Monod to hypothetical enzyme targets for opioid drugs. A cartoon depicting their proposed mechanism underlying tolerance and dependence induced by opioids is presented in Fig 3. The drug target is presumed to be an enzyme whose product is a critical mediator of functions that are inhibited by the opioid drug (neither Shuster nor

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the Goldsteins proposed a specific target enzyme). In the absence of opioid, the enzyme is active, generating a product that leads to functional activity in the system. A second property of the enzyme product is inhibition of synthesis of the critical enzyme by activating the repressor pathway identified by Jacob & Monod. In the presence of opioid, product levels decline, and repression of enzyme synthesis is removed, resulting in increased enzyme activity and partial reversal of the opioid drug effect. In pharmacological terms, tolerance has developed. If the drug is removed at this point, the activity of the de-repressed enzyme returns to above baseline levels and the system becomes hyperactive; withdrawal symptoms that are the opposite of the acute actions of the drug are then exposed. Reapplication of the drug immediately inhibits the enzyme, product levels fall back to normal levels and the withdrawal symptoms are reversed.

A more physiologically based model that applied similar concepts to the function of neural synapses was advanced by Sharpless (1964) and by Collier (1965; 1969). They proposed changes in the levels of neurotransmitter receptors as a result of chronic opioid exposure, rather than the undefined enzymes implicated by Shuster and the Goldsteins. Chronic opioid exposure would result that increased synthesis of neurotransmitter receptors above baseline levels. The effects of the drug would be observed as initial inhibition of neural activity, but with prolonged drug exposure increased receptor synthesis would result in tolerance to the drug effect, and removal of the opioid would result in hyperactivity of down-stream effector systems manifest as the expression of withdrawal symptoms. This would be analogous to the post-synaptic neural supersensitivity that was observed to follow damage to an upstream neuron. The models proposed independently by Sharpless and Collier were conceptually very similar; Fig 4 presents a cartoon depicting their essential features. Initial support for conceptual models proposing a requirement for increased enzyme or receptor synthesis in the development of opioid tolerance and dependence was provided by the observation that protein synthesis inhibition prevented the development of tolerance and dependence on opioids in experimental animals (Cox et al, 1968; Way et al, 1968). It is

now known that extensive changes in gene expression and protein synthesis occur during chronic opiate exposure and withdrawal (Nester, 1997).

These experimental models of drug dependence and addiction also had an impact on treatment approaches. If the underlying cause of tolerance and dependence was an adaptation in the central nervous system caused by chronic exposure to the opiate drug, it was possible that a critical endogenous regulator was now suppressed in some way, requiring replacement with an exogenous agent. Vincent Dole and colleagues proposed a novel approach to the treatment of addiction on opiates based the use of methadone in the form of a maintenance therapy to suppress withdrawal symptoms (Dole & Nyswander, 1965; Dole et al, 1966). These studies and further developments in the treatment of opioid addiction are considered in more detail in another article in this journal by Mary Jeanne Kreek, one of the original authors of the first methadone maintenance treatment program.

Conclusions:

By the late 1960s, recent developments in understanding opiate actions had answered some questions, but raised many more. Advances in neurobiology and pharmacology had made it possible to conduct mechanistic studies of drug effects on the nervous system. Several groups were initiating studies seeking to identify the proposed receptors for opiate drugs and others were exploring the bases of drug tolerance and dependence. When the founders of INRC met together during the 1969 World Congress of Pharmacology in Basel, Switzerland, the need for a forum for the presentation and discussion of the rapidly accumulating new results in this area of research was very apparent, and the Founders were willing to take on this task. The establishment of INRC as an annual conference at which the latest developments could be reported and discussed in a critical but friendly environment contributed significantly to the growth of interest in the field, to the development of multidisciplinary collaborations between laboratories, and across continents. It also fertilized the growth of related fields – for example, the explosion of interest in the study of neuropeptides in general, and in the creation of a comparable organization promoting research on cannabinoids.

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Author Contribution:

- o participated in research design
- o conducted experiments
- o contributed new reagents/analytical tools
- o performed data analysis
- X wrote the manuscript

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Footnote:

The opinions expressed in this article are the views of the author and should not be construed to reflect the position of the Uniformed Services University or the Department of Defense.

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Figure Legends

Fig 1. Cartoons of the Opioid Receptor Surface by Beckett &Casy (1954) and Portoghese (1965). Panel A. Diagrammatic representation of the proposed “analgesic receptor surface”; XII is levorphanol, XIII is dextrophan, XIV represents the proposed receptor surface; a detail from Fig 4 of Beckett & Casy (1954) (reproduced with permission from the Journal of Pharmacy & Pharmacology); Panel B. Schematic illustration of different binding locations on the postulated “analgesic” receptor surface for two different ligands. The protonated amine nitrogen is represented by + and the square demotes an N-substituent. The anionic site of the proposed receptor lies directly beneath the +. From Portoghese (1965) (reproduced with permission from the Journal of Medicinal Chemistry).

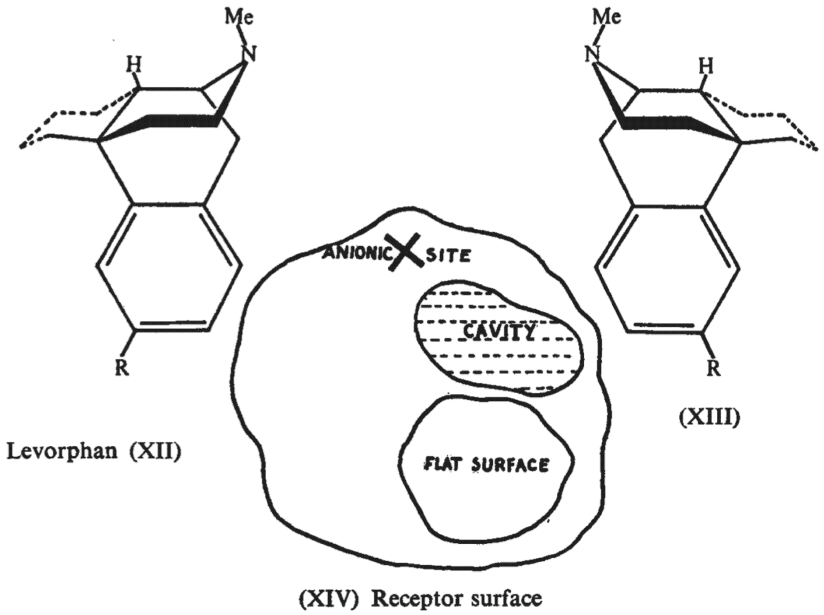
Fig 2 The effects of an opiate drug on the responses of the isolated guinea-pig ileum preparation to electrical stimulation and to acetylcholine. This is Fig 13 from Paton (1957; reproduced with permission from the British Journal of Pharmacology) in which he demonstrated that morphine reduced the contractions of the longitudinal muscle induced by electrical stimulation (applied at 6 stimuli/min) but had no effect on the response of the preparation to applied acetylcholine (ACh; amount indicated in µg), leading Paton to conclude that morphine was reducing the release of ACh from the neuronal myenteric plexus in the ileum preparation but not affecting the action of ACh on its receptors on the smooth muscle. The bath fluid was changed after each dose of ACh.

Fig 3. Cartoon depicting the proposed role of enzyme inhibition and repression in the development of tolerance to and dependence on opiate drugs, based on the very similar models of Shuster (1961) and Goldstein& Goldstein (1961; 1968). In this model the drug target is assumed to be an enzymatic reaction in which the production of active product leads to a functional response - in considering the actions of opiate drugs, the Function depicted in the Figure might be recognition of a nociceptive stimulus. The enzymatic product not only drives a functional outcome but also exerts a feedback repression of the synthesis of the enzyme, preventing excessive activation of the

system. The acute action of the opiate drug is to inhibit the enzymatic reaction, leading to reduced levels of the active product and acutely inhibiting detection of the nociceptive response, but also reducing the feedback repression of enzyme synthesis. Over time after repeated opiate drug exposure, enzyme levels become elevated thus partially overcoming the inhibition of active product generation by the drug and reducing its ability to inhibit the nociceptive stimulus (i.e, tolerance is induced). If the drug is abruptly removed, the presence of higher levels of enzyme, relative to baseline levels, results in an exaggerated nociceptive response (i.e., a withdrawal reaction occurs) that will persist until repression of enzyme synthesis reduces the amount of enzyme present back to baseline levels, at a rate that is determined in part by the rate of degradation of the enzyme.

Fig 4. A cartoon depicting the basic features of the proposals by Sharpless (1964) and Collier (1965) based on then current understanding of the phenomenon of denervation supersensitivity. Acutely, the drug (e.g., an opiate) reduces the release of transmitter, producing an initial agonist action. During chronic dosing, the reduced activation of post-synaptic receptors as a result of reduced release of transmitter is proposed to lead to increased expression of these receptors on the post-synaptic neuron, leading to tolerance to the initial action of the drug. When chronic drug administration is stopped (withdrawal) the release of transmitter recovers quickly but it takes much longer for the number of post-synaptic receptors to revert to basal levels. During this period the down-stream response is exaggerated, resulting in the expression of withdrawal symptoms.

A.



B.

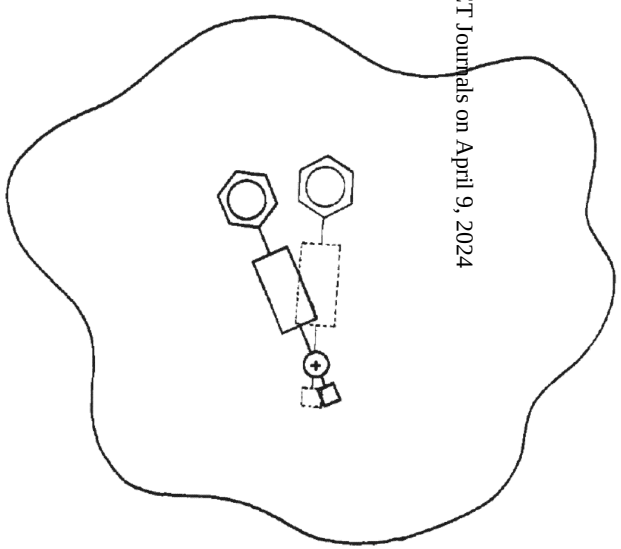


Fig 1.

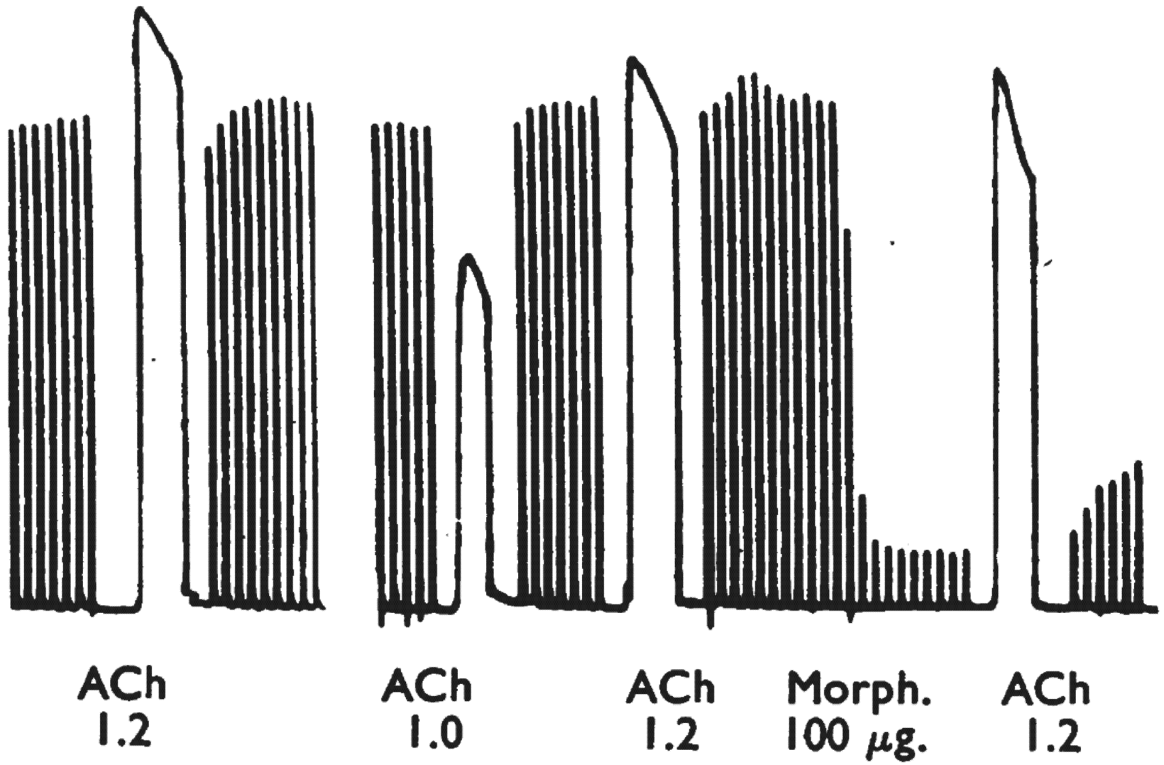
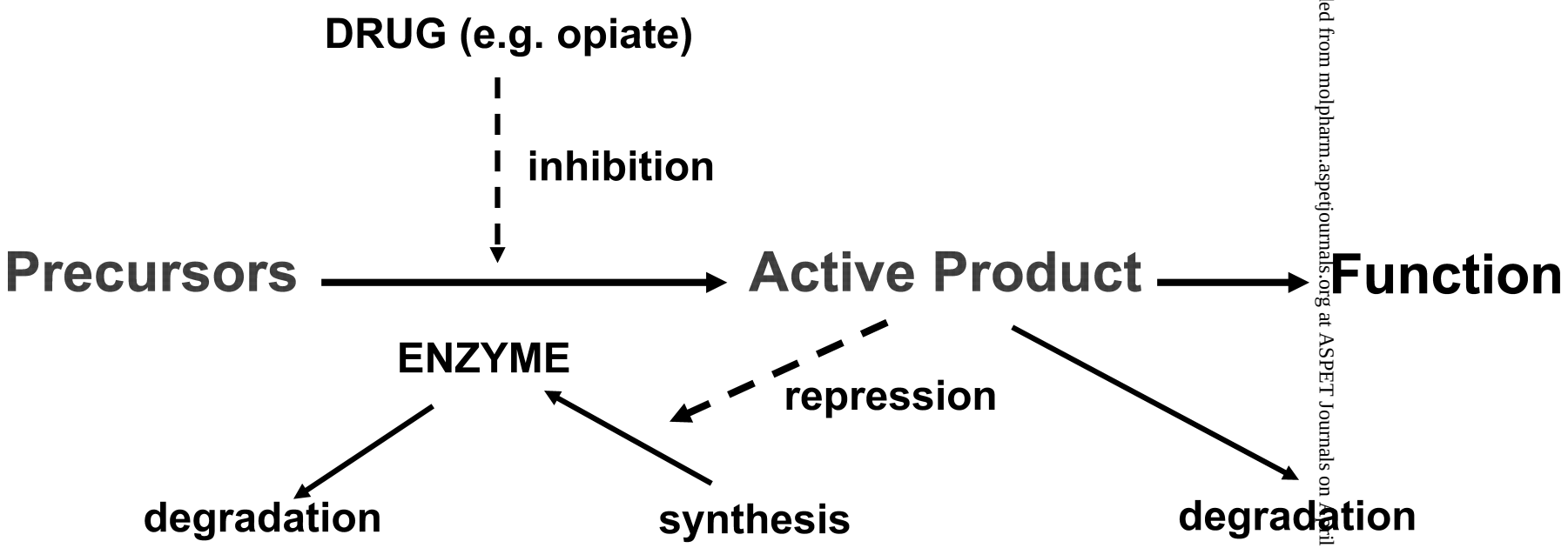


Fig. 2



- Acutely, the opiate drug inhibits the enzyme reaction, reducing the level of active product (resulting in, for example, antinociception)

- After chronic drug exposure:
  - reduced levels of the active product removes repression of enzyme synthesis
  - increased enzyme synthesis results in increased activity, counteracting the drug effect (i.e., tolerance occurs)

- After withdrawal of drug:
  - enzyme inhibition by the drug declines rapidly as the drug is eliminated
  - enzyme levels decline more slowly - excess active product produced (leading to a withdrawal reaction).

Fig 3

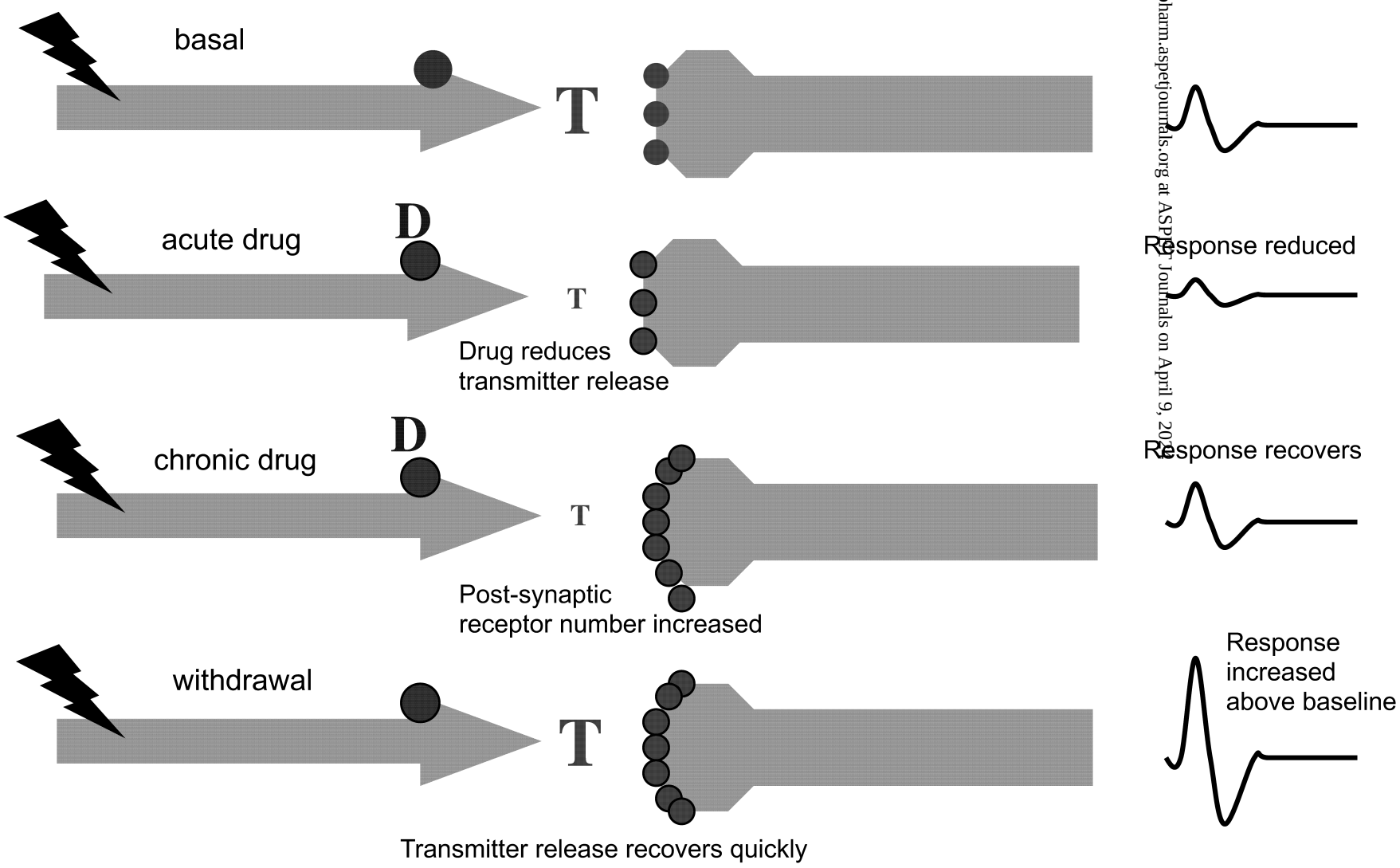


Fig 4.