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Nicotine, alcohol and cocaine coupling to reward processes via endogenous morphine signaling: The dopamine-morphine hypothesis

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George B. Stefano[1], Enrica Bianchi[2], Massimo Guarna[3], Gregory L. Fricchione[4], Wei Zhu[1], Patrick Cadet[1], Kirk J. Mantione[1], Federico M. Casares[1], Richard M. Kream[5], Tobias Esch[6,7]

[1] Neuroscience Research Institute, State University of New York College at Old Westbury, Old Westbury, NY, U.S.A.
[2] Department of Neuroscience, University of Siena, Siena, Italy
[3] Department of Biomedical Sciences, University of Siena, Siena, Italy
[4] Division of Psychiatry and Medicine, Massachusetts General Hospital, Boston, MA, U.S.A.
[5] Department of Biochemistry, Downstate Medical Center, State University of New York, Brooklyn, NY, U.S.A.
[6] Division of Integrative Health Promotion, Coburg University of Applied Sciences, Coburg, Germany
[7] Institute for Integrative Medicine and Health Research, Potsdam, Germany

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Summary

Pleasure is described as a state or feeling of happiness and satisfaction resulting from an experience that one enjoys. We examine the neurobiological factors underlying reward processes and pleasure phenomena. With regard to possible negative effects of pleasure, we focus on addiction and motivational toxicity. Pleasure can serve cognition, productivity and health, but simultaneously promotes addiction and other negative behaviors. It is a complex neurobiological phenomenon, relying on reward circuitry or limbic activity. These processes involve dopaminergic signaling. Moreover, nicotine, cocaine and alcohol appear to exert their pleasure providing action via endogenous morphinergic mechanisms. Natural rewarding activities are necessary for survival and appetitive motivation, usually governing beneficial biological behaviors like eating, sex and reproduction. Social contacts can further facilitate the positive effects exerted by pleasurable experiences. However, artificial stimulants can be detrimental, since flexibility and normal control of behavior are deteriorated. Additionally, addictive drugs are capable of directly acting on reward pathways, now, in part, via endogenous morphine processes.

key words: **pleasure • reward • addiction • motivation • nicotine • ethanol • dopamine • morphine**

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Author's address: Dr. George B. Stefano, Neuroscience Research Institute, State University of New York – College at Old Westbury, Old Westbury, New York, 11568, U.S.A., e-mail: gstefano@sunynri.org

BACKGROUND

The biological mechanism mediating behavior which is motivated by events commonly associated with pleasure is called 'reward'. It may govern normal behavior through pleasurable experiences [1]. Pleasure is a subjective phenomenon, i.e., subjective quality. Hence, an intimate association between reward and pleasure exists [1,2]. Pleasure or reward is found in motivation circuitries imbedded in the central nervous system (CNS). Anatomically, these pathways are particularly linked to the brain's limbic system.

When biologists (or psychologists) talk about motivation, behavior, reward and, particularly, pleasure, they usually relate to vertebrates or mammals, including humans. It has been suggested to use the term 'reinforcement' instead to characterize comparable behaviors and biological strategies in primitive animals, or the general biological principle involved, thereby avoiding linguistic vagueness and even confusion, yet emphasizing the fact that reward and pleasure are regarded as highly subjective experiences ("feelings"), i.e., interpretations, due to higher organizational levels [3,4]. Also, primitive animals like invertebrates don't possess a brain, and therefore don't have a mammalian-like limbic system. However, when neurobiologists adopt a more distant position they find surprising analogies and similarities [5,6]. Especially, the molecular and physiological structures and substances involved in reinforcement and reward, including the specific receptors used, are largely identical. Further, the autoregulatory systems activated in motivational signaling have been conserved during evolution, thus they are, in a biological sense, extremely conservative, as we and others have shown for the endocannabinoids and opioid peptide as well as opiate alkaloid signaling systems [7–9]. Thus, with this work, we will outline and examine the common and underlying organization of reward and pleasure phenomena to form our hypothesis, rather than focusing upon the undeniable differences in motivation biology, e.g., with reference to genus.

Motivation may be divided into two categories, appetitive and aversive motivation [6]. Appetitive motivation concerns behavior directed towards goals that are normally associated with positive hedonic, i.e., pleasurable, processes (food, recreational drugs, sex, etc.). In contrast, aversive motivation involves getting away from hedonically unpleasant conditions [1]. Pain and pleasure conditions, in specific situations, have the capacity to serve survival. The physiological substrate for reward and avoidance primarily lies within the limbic system [10–14].

Neurobiologists have long known that the euphoria induced by drugs of abuse, sex or other stimuli arises because of complex circuits of nerve cells that evolved to make us feel good and they are related to survival and passing along our genes [15–17]. Reward pathways are evolutionarily ancient [5]. A crucial component of CNS reward and motivation circuitries are nerve cells that originate in the ventral tegmental area (VTA), near the base of the brain. These cells send projections to target regions in the frontal brain, most notably to a structure deep beneath the frontal cortex, (i.e., nucleus accumbens) [15,16]. The essential neurotransmitter of this connection is dopamine. In mammals (humans), the reward circuit is more complex, and it is integrated with several other brain regions that serve to enrich an experience with "emotion" and direct the individual's response or behavior toward rewarding stimuli, including food, sex and social interaction [2]. The amygdala, for instance, is a special part of limbic and reward systems that is closely related to emotion (especially fear) and has post-synaptic receptors for inhibitory gamma-aminobutyric acid (GABA) [5]. Diazepam and other anxiolytics mimic the action of GABA at this site. Recent findings also link endogenous morphine production to these limbic structures [18–21].

The amygdala also helps to assess whether an experience is pleasurable or aversive (and whether it should be repeated or avoided) [15,16]. Meanwhile, the hippocampus participates in recording memories of an experience, including where, when and with whom it occurred [2]. The frontal cortex, however, coordinates and processes all this information and consequently determines the ultimate behavior. Finally, the VTA-accumbens pathway acts as a measuring tool and regulator of reward: it 'tells' the other brain centers how rewarding an activity is [2]. The more rewarding an activity is deemed, the more likely the individual is to remember it well and repeat it [2].

With regard to frequent neuronal reward 'tracks' within the CNS, activation of the medial forebrain bundle (MFB), as it courses through the lateral hypothalamus to the ventral tegmentum, produces robust rewarding effects [1,22]. Again, dopamine is involved [23]. Electrophysiological and neurochemical techniques revealed: CNS stimulation may activate a descending component of the MFB which is synaptically coupled at the ventral tegmentum to the ascending mesolimbic dopamine system, i.e., nucleus accumbens [1,2,22–24]. Pleasure-inducing electrical stimulation thus involves a circuitous reward pathway, first activating a descending MFB component and then the ascending mesolimbic dopamine pathway. Clearly, we can speak of brain's reward and motivation circuitries.

Taken together, the brain possesses multifunctional pathways that mediate pleasure, reward and motivation. Psychomotor stimulants and exogenously administered opiates, similar to experimental electrical stimulation, activate this reward system by their pharmacological actions in the VTA and nucleus accumbens [15,16,24]. Ventral tegmental activation, however, as well as other essential CNS reward features, involves dopamine signaling. Other neurotransmitters (e.g., GABA, glutamate, serotonin, stress hormones) may play a critical role too [6,25,26]. Natural rewards like food and sex in accordance with other substances, such as caffeine, ethanol, nicotine, may also activate brain's reward and motivation circuitries [6,24,27].

ADDICTION

Reward and motivation can be considered a natural component of normal behavior as noted earlier. Clearly, reward pathways serve to direct behavior towards goals that are normally beneficial and promote survival of an organism or species, e.g., food and water intake, reproductive activities [17,28–30]. These generally pleasurable actions are useful and may not constitute addiction. However, a loss of flexibility and the disability to make free decisions, i.e., extreme control of behavior, as described in addiction, may be seen

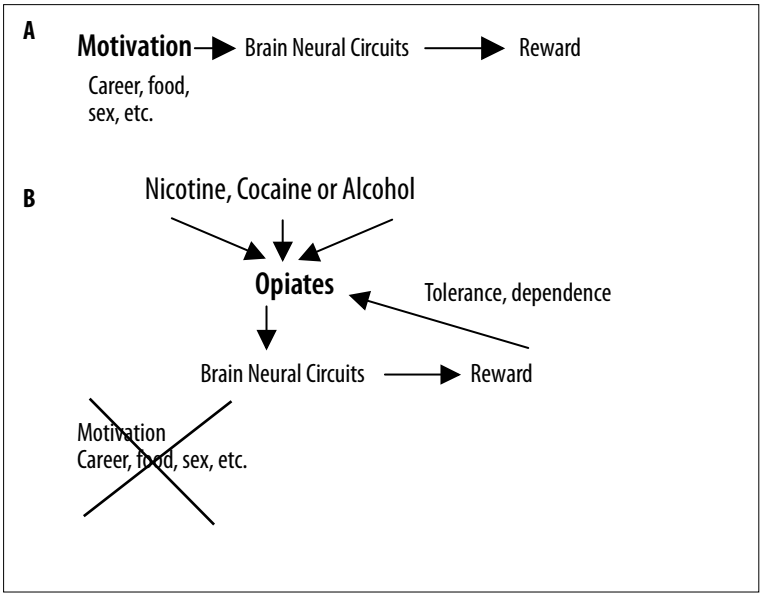

Figure 1. Individuals have conventional motivation items that depend on brain neural circuits as described in the text (**A**). Once these motivational items are achieved such endowed individuals feel good, i.e., reward processes. These processes in part depend on opioid and opiate signalling. If these chemical messengers are taken exogenously (**B**), normal motivational stimuli are lost since the same result can be achieved by bypassing these processes. Recently, it was demonstrated that other substances having the potential for abuse (nicotine, alcohol and cocaine) work in part by enhancing the release of endogenous morphine, adding a commonality factor to this complex phenomenon. However, because high doses of these chemical messengers can be taken these neural circuits become tolerant to these substances, and by the circuits nature the individual wants to experience more artificial reward process activity, leading to higher doses and eventual dependence. Thus, substances of abuse create a short-circuit, producing reward activity at a level that was never meant to be. We surmise because this short-circuit requires not only adaptations by physiological process but molecular modifications, that some of the resulting CNS changes may be permanent.

as one of the distinguishing features between pleasure/reward and addiction [6]. This addiction-related behavioral inflexibility and the impossibility of normal rewards to govern behavior has been refereed to as *motivational toxicity* by Bozarth [1].

In biology, drugs of abuse are able to act as 'positive reinforcers' (see above), meaning that they are stimuli able to promote an increase in the probability of responses on which they are contingent. This ability does not necessarily rely on a hedonic property of these substances [31]. However, the molecular basis of these responses obviously lies within the autoregulatory signaling systems, e.g., reward circuitries [10,32], still allowing for scientific dispute upon the correct use of terms like 'reward' and 'pleasure' in association with drug administration in non-human animals [33]. Even more, pleasure, reward, reinforcement and instrumental learning, although they all possibly relate to positive motivational or behavioral responses in animals and humans, may, in a biological sense, not all be the same [33–35]. For our hypothesis, however, these biological or, depending on the specific approach chosen, semantic distinctions appear to be of minor interest, since the common and underlying neurobiology seems to allow us to infer and form our more generally admitted molecular hypothesis, as stated in the title of this work. Clearly, drug ingestion influences these dopamine-morphine pathways and physiology (see below).

Some drugs, like cocaine and heroin, quickly and uniformly exert extreme control over behavior, while others are less potent, such as moderate alcohol consumption or occasional nicotine or marijuana use [1]. The most powerful drug rewards include the psychomotor stimulants (e.g., amphetamine, cocaine) and opiates (e.g., heroin, morphine). Indeed, drug influence on behavior not only depends on the amount, duration and frequency of abuse but also on the type of substance involved and the environment [15,16,36,37]. Thus, personality, social and genetic factors, in addition to individual differences in reward or motivation system functioning and physiology, may also play an important role [1,2,5,10,38–49]. Taken together, addiction may be characterized by a loss of control over pleasurable and biologically useful events ('healthy drug use'), turning a positive motivation into a disaster.

In creating this artificial state of pleasure some chemicals bypass the sensory receptors mediating natural rewards (see above; Figure 1) [5,6]. In fact, caffeine, alcohol and nicotine, given as examples, all activate brain reward pathways directly. This raises a major question, how do they do this? Moderate use of these substances (especially alcohol) has gained widespread acceptance. Moreover, low-dose consumption sometimes is considered healthy [50–53]. Further, some drugs are known for their recreational use, involving, for instance, desirable psychological effects, such as relaxation and stress reduction [5,14,39]. Much like moderate caffeine and alcohol use, potent addictive drugs activate brain reward systems directly. But this activation may also be much more intense, causing the individual to crave the substance and focus activities solely around drug ingestion [1,2]. Thus, the ability of addictive drugs to strongly activate CNS reward systems and to chemically alter normal functions of these systems is a crucial feature of addiction [1,38,54–57].

With regard to neuropathophysiological mechanisms supporting addiction, we find uniform signaling pathways and common CNS activities underlying different forms of drug abuse and different subjective experiences. At the bottom, as described, lies limbic and reward system stimulation, in-

cluding VTA, extended amygdala and prefrontal cortex activity. However, addiction is a complex phenomenon. Immediately after drug ingestion, feelings of pleasure, euphoria, and rush predominate (the sublenticular extended amygdala and VTA are of particular importance here), followed by induction of craving with accentuated amygdalar and nucleus accumbens activity [15,16]. The craving grows as the euphoria wears off. Moreover, initial drug exposure triggers tolerance and, in the drug's absence, discomforts that only more drugs can cure [2]. Tolerance and dependence are related to a suppression of the brain's reward circuitry that, ironically, is a key feature of frequent and continued drug abuse [2,15]. Thus, the reward system fails to give rewards in the end. The situation changes, however, when drug consumption is stopped for a longer period of time. But the neurophysiology then does not necessarily return to normal, since relapse vulnerability stays and may even grow bigger. Ultimately, following a 'successful' withdrawal, drug sensitization may take over [15,16,58]. This secondary effect of drug consumption, i.e., addiction, is associated with a characteristic pattern of cellular gene and protein production.

Briefly, there is a growing body of evidence regarding intracellular cascading actions of addicting compounds. When drugs are ingested, dopamine levels especially in the nucleus accumbens rise, stimulating dopamine-responsive cells to enhance cyclic AMP (cAMP) concentrations, activating CREB (cAMP response element-binding protein) [2,59,60]. CREB induces a specific gene expression, coding for proteins that, for example, suppress the reward circuitry (i.e., tolerance induction) [15,16,60]. One of these CREB-dependent proteins is dynorphin, a natural molecule with opium-like effects, that is synthesized in the nucleus accumbens and triggers a negative feedback loop, exerting inhibitory effects on VTA neurons [2,24,61]. The increase in dynorphin also facilitates dependence, since its reward suppression leaves one depressed and unable to take pleasure in previously enjoyable activities (in the drug's absence) [2,61]. Although such loss of interest in previously pleasurable activities has been reported in human studies, some studies in rodents have found opposite results, again demonstrating the complex physiology behind these phenomena and therefore, this possible link between dynorphin and mood disturbance is still a matter of contrarian scientific debate [62–64]. Furthermore, CREB is switched off only shortly after drug consumption has ended. Thus, this transcription factor may not be responsible for conditions that draw 'former' addicts back to substance abuse after years of abstinence. Such relapse is driven, for example, by drug sensitization, a phenomenon that sets in when drug use stops and tolerance wanes [15,16,60]. Delta FosB, a transcription factor that exerts its functions in response to chronic drug abuse, is released in the nucleus accumbens [60,61,65]. This stable protein remains active for months following drug ingestion, possibly controlling gene expression even after the cessation of drug taking [2,60]. Hence, delta FosB may cause drug addicts to become hypersensitive to drugs, leading to relapse even when only minimal doses of drugs are encountered [15,60,65]. Interestingly, it is also induced in response to repetitious non-drug rewards and may therefore represent a more general mechanism participating in reward-associated behavior change [2,15,16]. Sensitization suggests that certain rewards are especially wanted by an organism, including useful (i.e., beneficial) and plainly pleasurable activities.

Many questions with reference to FosB, dynorphin and CREB activation still remain open. For example, experimental conditions that lead to CREB activation in the nucleus accumbens and dynorphin upregulation also enhance enkephalin, a condition that has previously been thought and discussed to have opposite effects on mood regulation [60,66–68]. Thus, the exact pathways and processes yet have to be determined and then put into the broader picture, since the final outcome of accumbal CREB-related changes still is unclear.

Taken together, reward circuitry alterations in the course of pleasure-seeking behavior and drug abuse potentially promote tolerance, dependence, craving, relapse and vulnerability [2,16,60]. Pleasure is a substantial feature of drug consumption and prolonged abuse facilitates tolerance and dependence. Due to CREB activity, sensitivity to the drug is reduced at first [2,60]. Yet, with more prolonged abstention, changes in delta FosB activity and glutamate signaling predominate [15,16,60,61,65]. These actions may trigger relapse by increasing sensitivity to the drug's effects (if used again), eliciting powerful responses to memories of past highs and cues that bring those memories to mind [2,15,58].

DOPAMINE

Dopamine is regarded as a key player in pleasure and reward physiology [6]. Although substantial parts of the brain's reward and motivation circuitry have already been discovered in the 1950's, it took over 20 years to apply this knowledge to the study of mechanisms involved in the rewarding aspects of substance abuse [69]. Within this context, opiate antagonists are capable of blocking the dopamine agonist activation of the CNS reward system [69]. Opioid receptors and their endogenous ligands are necessary for dopamine action on the reward system. Thus, dopamine and endogenous opiates/opioid peptides appear to be interconnected in the drug-pleasure-reward chain [69,70]. Yet, dopamine may represent a basic link of this chain.

Although dopamine and its CNS systems seem to be a key player in reward, pleasure, and even placebo physiology [6,71], evidence exists showing that critical parts of these neural systems get activated by different kind of stimuli, regardless of their hedonic or pleasure value, at least at first sight. For example, dopamine release is seen in response to pleasurable but also to painful or even novel stimuli [72]. However, maybe stress is of importance here and/or the expectation of a specific outcome, i.e., positive (but not feeling absolutely confident about it), together with an attitude of curiosity, that triggers higher dopamine levels, since these characteristics are linked to dopamine release [73,74]. Thus, a direct hedonic value must not be the primary aspect or goal of reward- or motivation-related CNS dopamine release.

Dopamine has received special attention from psychopharmacologists, due to its obvious role in mood, affect and motivation regulation [1,24,59]. Also, stress regulation and social behavior depend on dopamine physiology, since, for example, higher dopamine concentrations (i.e., in the brain) lead to greater aggressiveness and elevated social status in ver-

tebrates [75–77]. Although several distinct dopamine systems (i.e., receptors and subtypes, functional circuits) exist in the brain, the mesolimbic appears to be the most important for motivational processes [1,61]. Many drugs seem to exert their effects on behavior by stimulation of the mesolimbic dopamine activity [1,2,58]. Drugs of abuse cause the nucleus accumbens to receive a flood of dopamine [15,16]. Cells in the mesolimbic dopamine system, however, show spontaneous activity – that is, action potentials are constantly generated at a slow rate. The result is a steady (basal) release of small amounts of dopamine into the synaptic cleft, maintaining normal affective tone and mood [1,78,79]. Moreover, some forms of clinical depression may result from unusually low dopamine levels [1,12,80], which also may be associated with drug consumption, since repeated use of cocaine or morphine, for example, may deplete dopamine from the mesolimbic dopamine system and reward circuitry [1,2,15]. These dopamine depletions may be responsible for normal rewards to lose their motivational significance (i.e., tolerance induction, motivational toxicity). At the same time, the mesolimbic dopamine system becomes even more sensitive to activation by psychomotor stimulants and opiates. Consequently, sensitization develops (as illustrated). Counter intuitively, abstinence from cocaine or morphine after repeated administration may also decrease dopamine levels in the mesolimbic dopamine system/VTA [81,82]. This deteriorated dopamine function may be related to the intense craving associated with withdrawal in human drug addicts [1]. Taken together, initial or incidental drug use may enhance dopamine output and increase the resulting feelings of pleasure. Over time, however, this physiological reward function may collapse, causing dopamine concentrations to drop down and possibly leading to depression and other negative affective states.

Various addictive drugs share the common feature of stimulating the same dopaminergic brain reward system (for example, heroin enhances dopamine levels by increasing dopamine release, whereas cocaine inhibits the dopamine reuptake), and this action has been related to their appetitive motivational effects [1,2,83]. Thus, appetitive rather than aversive motivation may induce drug-taking behavior and addiction [84]. Reward processes, based on dopaminergic signaling, clearly exhibit a positive motivational potential and with that they may be useful for medical strategies focusing on behavior change, i.e., stress management and lifestyle modification programs (see below).

VTA neurons communicate with the nucleus accumbens by dispatching dopamine from the terminals of their long projections to receptors on nucleus accumbens neurons [15,16]. This dopamine pathway from VTA to nucleus accumbens appears to be a critical component of the reward physiology, including pleasure and addiction: in animals with lesions in these regions, a loss of interest in drug consumption has been observed [2,58]. Moreover, cocaine and other stimulants temporarily disable the return of dopamine to the VTA neuron terminals and opiates, in addition, bind to inhibitory neurons in the VTA that usually shut-down the dopamine production (thereby allowing dopamine-secreting cells to release dopamine). Both strategies finally support excess dopamine to act on the nucleus accumbens [15,16]. Opiates may further generate a strong 'reward message' by acting directly on the nucleus accumbens [2,70]. Again, we find the complexity that rules dopaminergic reward processes.

When drugs of abuse increase dopamine release from the VTA into the nucleus accumbens, they further alter the responsiveness to glutamate [2,15,16]. Changes in sensitivity to glutamate may then enhance both the release of dopamine from the VTA and responsiveness to dopamine in the nucleus accumbens, thereby promoting CREB and delta FosB activity (see above). Furthermore, it seems that this altered glutamate sensitivity strengthens the neuronal pathways that link memories of drug consumption and related cues with high reward, thus feeding the desire to seek the drug, i.e., vicious circle [15,16,58]. Finally, drugs of abuse obviously influence the shuttling of glutamate receptors in the reward pathway [2].

Linking the dopamine hypothesis to opiate alkaloid endogenous system has been substantiated in recent years because it has now been demonstrated that animal tissues and whole animals, including human, can make morphine [85–87]. Simultaneously, on a nervous system level these findings then place endogenous morphine in the mental health field, transcending analgesic and reward processes. It also appears likely that functional bases exist that facilitate coupling of a variety of drugs of abuse in reward and pain pathways to dopamine and endogenous morphine expression [82,88–93]. The alteration of dopamine functions, especially mesolimbic, appears to be a common factor for many substances of abuse [82,88–93]. Dopamine serves as a major precursor of endogenous morphine [85–87]. Therefore, dopamine metabolism alteration may induce modifications in morphine synthesis and consequently weaken endogenous morphine's action on the "dopamine" reward system.

MORPHINE SIGNALING

Recently, normal healthy human white blood cells (WBC) and invertebrate neural tissues were found to have the ability to synthesize morphine, opening up a new world of comprehension concerning endogenous morphine processing and signaling [85,86]. Human morphine synthesis was demonstrated in human white blood cells (WBC), specifically polymorphonuclear neutrophils cells (PMN), from its precursors, L-DOPA, reticuline, THP and tyramine, in a concentration-dependent manner [86]. Furthermore, we identified CYP2D6 as a major enzyme regulator in this pathway [86]. CYP2D6 appears to act at critical steps of the morphine biosynthetic pathway in PMN [85,86,94–99]. We also demonstrated that bufuralol, a CYP2D6 substrate, as well as a CYP2D6 competitive substrate, diminished morphine synthesis in PMN exposed to tyramine. This provides additional support for the ability of this tissue to synthesize morphine via dopamine since we and others have shown that this enzyme is expressed in PMN [56,87,99,100]. Interestingly, CYP2D6 is also involved with morphine synthesis in *Mytilus edulis* pedal ganglia, demonstrating the conservation of this mechanism in organisms 500 million years divergent in evolution [85]. Therefore our data show that a key enzyme family, which is also present in mammalian brain, is expressed in WBC and is capable of synthesizing morphine via tyramine.

The CYP2D6 isoenzyme, found on chromosome 22, is part of the CYP2 family and is expressed in human neural, im-

mune and hepatic tissues [101]. CYP is involved with the metabolism of many endogenous compounds such as biogenic amines, steroids, leukotrienes, fatty acids and prostaglandins, where it may generate inactive or active compounds [101]. The CYP system is involved in the oxidative metabolism of pharmaceutical compounds as well. For example, anti-arrhythmics, beta-blockers, antidepressants, anti-psychotics, vasodilators and, as demonstrated in the present report, analgesics are also substrates for this enzyme [101].

In regard to dopamine, the morphine-enhancing effect of L-DOPA and its key position in animals, including man, in both the dopamine and morphine biosynthesis pathways suggest that morphine may be involved in a regulatory step controlling its own synthesis [56,94]. The observation that L-DOPA can also lead to increased PMN morphine concentrations suggests that an additional pathway besides that originating from tyramine for morphine synthesis exists via tyrosine [56,85–87]. It is important to note that WBC (i.e., both B and T lymphocytes) also contain dopaminergic signaling components [102–104], further supporting this hypothesis. Furthermore, dopamine can significantly affect the immune system [reviewed in [105]]. Depending on the environment (*in vitro* vs. *in vivo*) and cell type, dopamine has activating and suppressive effects on cytokines such as IL-1β, IL-2, IL-6, TNF and interferon. How these effects are mediated is unknown, however our data suggest a potential role for morphine signaling in some of these processes [106,107].

In addition to a direct metabolic link, there are alternative hypotheses regarding the interactions between dopamine and morphine. For example, dopamine may be acting via cell surface dopamine receptors, supported by the observation that dopamine receptor antagonists can block morphine-induced immunomodulation [108]. The time course for these observed changes is likely much slower, since dopamine is a morphine precursor, than the metabolic link between dopamine and morphine that we have described in this study.

In summary, several data support the coupling of the morphine and dopamine biosynthetic pathways [7,87], a finding that has tremendous biomedical significance that crosses many physiological systems. Further studies that more clearly elucidate the potential interplay between these two regulatory chemical messengers and their effects on immune, neural and vascular systems are currently under way.

NICOTINE

In a somewhat parallel story with opiate alkaloid induced addiction [6], nicotine also is addictive [109]. As with morphine, it exerts profound cellular and synaptic actions that modulate motivational and behavioral changes that can be associated with addiction [6,21,109–112]. It is known that patients with schizophrenia who are on neuroleptic dopamine receptor blocking medications readily develop nicotine addictions. With a decrease in the availability of D2 receptors, cigarette smoking in schizophrenic patients increases [113].

As noted earlier, we have recently demonstrated that morphine can be made by invertebrate ganglionic tissues and human white blood cells in a process mediated, in part, by CYP2D6, a CYP 450 isoform [85,86]. Again, this parallels the nicotine metabolic pathway that is mediated by a CYP 450 isoform, namely CYP2A6 [114]. Nicotine is C-oxidized to cotinine, which is catalyzed by CYP2A6 [114]. Calcium cellular mechanisms are involved in both morphine and nicotine signaling [115,116]. On a comparative level, as noted earlier, nicotine stimulates dopamine presynaptic release [117], accounting for the drop in morphine levels after the initial increase in *M. edulis* ganglia [27]. This further suggests that acetylcholine may stimulate morphine presynsaptic release. Furthermore, nicotine may lead to elevated CNS dopamine levels in balance (boost effect), as experienced, e.g., by smokers [118]. Both morphine and nicotine appear to share the property of immunosuppression [119–125].

Comparatively, exposing *M. edulis* pedal ganglia to reticuline increases endogenous ganglionic morphine levels [126]. L-DOPA injection into normal and healthy *M. edulis* has also resulted in an increase in ganglionic morphine levels [127] as it does in human tissues [86]. In addition, these animals contain norlaudanosoline (tetrahydropapoverine), another precursor in the proposed animal morphine biosynthetic pathway [128]. As in humans, CYP2D6 is involved in this synthesis [85,86]. *M. edulis* also exhibits cholinergic signaling. Nicotine in a receptor mediated manner also can enhance and depress endogenous morphine levels, acting biphasically [27]. Mollusks have muscarinic- and nicotinic-like receptors [129–132]. This same situation exists in *Mytilus edulis* [133]. These findings make *M. edulis* tissues an ideal model for examining the effects of various compounds on dopamine and morphine metabolism and signaling.

Additionally, nicotine does promote a statistically significant enhancement of ^{125}I-trace labeled morphine released from invertebrate ganglia into the extracellular medium in a concentration dependent manner [134]. The same has been shown to occur in human white blood cells [135], suggesting that nicotine's effects occur via an enhancement of endogenous morphine signaling.

In another study, it was demonstrated that nicotine mediates an unusually potent pharmacological effect on release of ^{125}I-trace labeled morphine from lobster nerve cord that is attributed to selective activation of invertebrate nicotinic receptors based on pharmacological inhibition by α-BuTx [136]. The observed pharmacological effects of nicotine were validated by a second series of drug trials employing the selective nicotinic receptor agonist epibatidine, albeit at concentrations 2–3 orders of magnitude higher than those realized with nicotine. Co-administration of nicotine (60 nM) and the pre-junctional ganglionic nicotinic antagonist hexamethonium (1 μM) produced a marked potentiation of ^{125}I-trace labeled morphine release. The novel potentiated response was also observed for epibatidine (35 μM) co-administered with the competitive nicotinic antagonist chlorisondamine, providing independent confirmation of putative nicotinic autoreceptor activity in lobster nerve cord. The muscarinic antagonist atropine at 1 μM promoted *ex vivo* evoked release of ^{125}I-trace labeled morphine, indicating the presence of a tonic cholinergic inhibitory tone mediated by separate populations of muscarinic receptors. The observed effect of ethanol to promote en-

hanced release of endogenous morphine was not affected by co-administration of α-BuTx at 1 μM, indicating that its pharmacological actions are not mediated by evoked release of acetylcholine via convergent activation of nicotinic receptors [136].

Excitatory effects of acetylcholine are mediated through muscarinic- and nicotinic-like receptors in invertebrate ganglia [129–133]. The presence of nicotinic receptors in invertebrates is supported by other studies in insects and lobsters [137–139]. This receptor, mediating nicotinic effects on endogenous morphine, is sensitive to α-BuTx binding and action as is also supported by the present study [137–139]. The fact that nicotine is 1000 times more potent than epibatidine suggests novel and highly selective nicotinic receptors mediating this phenomenon. Furthermore, nicotine is associated with nitric oxide release in insects [138]. The results of this and other recent studies [136] strongly suggest a sophisticated cholinergic interaction between muscarinic and nicotinic type receptors mediating the release of morphine, which also has been documented in *Aplysia* [140]. It also suggests tonic inhibition of this release as well as the presence of auto receptors mediating release [141,142]. Interestingly, this level of sophistication involving cholingeric receptors, including novel types and actions of inhibitors, is documented in the literature, including in invertebrates [140–143].

Taken within the context of the lobster study, as described, nicotinic signaling is coupled to endogenous morphinergic processes and associated behaviors in a very precise manner [136]. Chlorisondamine, a nicotinic antagonist, prevents nicotine from exerting its rewarding effect on the CNS [144]. Epibatidine, a nicotine agonist [145], can exert analgesic actions [146,147]. These mammalian observations are supported in the lobster study by the fact that nicotine and epibatidine release neuronal stores of morphine, accounting, in part, for the nicotinic induced analgesia and reward properties noted in mammals earlier [136]. This coupling is further enhanced by the fact that both compounds appear to release constitutive nitric oxide in a calcium dependent manner in mollusks [148]. Thus, it would appear that there is a significant amount of convergence between these signaling mechanisms, supporting our hypothesis that nicotine exerts its rewarding and addictive actions via endogenous morphine release, allowing it to become active and the actual signal behind these reward/pleasure/addictive processes.

It would appear that links may exist in these pathways, coupling these signaling molecules in reward and pain pathways, as well as others. The data taken as a whole demonstrate that nicotine enhances ganglionic morphine levels followed by a statistically significant decrease [27], suggesting this as a role for ganglionic acetylcholine. It also strongly suggests nicotine's addictive properties may arise from this ability to enhance endogenous morphine levels, opening up a new level of understanding in nicotine induced addiction and behavioral effects as well as morphine regulation.

ETHANOL

Zhu and colleagues (2006) demonstrate that exposing *M. edulis* pedal ganglia to ethanol results initially in significant increases in ganglionic morphine levels, which is time- and concentration-dependent [134]. This initial effect is followed by a significant lowering of endogenous morphine levels. They surmised in man that the ability to alter endogenous morphine levels may represent an underlying process whereby ethanol can provide pleasure and depression as well as its reported addicting properties. In *M. edulis* this may just represent a "rewarding" experience, leading to repetitive seeking of an activity linked to this process, feeding via controlling ciliary activity [149–153]. Furthermore, alcohol promotes a statistically significant enhancement of ^{125}I-labeled morphine released from invertebrate ganglia into the extracellular medium in a concentration dependent manner [134]. The same occurs in human white blood cells [135], suggesting that alcohol's effects occur via an enhancement of endogenous morphine signaling. These results are in congruence with that demonstrated for nicotine, as discussed earlier.

Supporting this conclusion are the studies that demonstrate alcohol is addicting and interacts with the reward system of the human brain, including exogenous morphine actions [154–158]. We now demonstrate that ethanol increases endogenous ganglionic morphine levels, as is the case for nicotine [27,134]. Thus, we surmise ethanol's addicting and short pleasure-promoting properties may be related to its morphine enhancing effect and its depressing effect to reducing neural morphine levels. Again, similarities between nicotine and ethanol on the neuroregulatory level become obvious.

Additionally, studies also link alcohol's effects with dopamine neural signaling. Microdialysis studies have shown that ethanol increases extracelluar dopamine concentrations in rat nucleus accumbens [159]. Tetrahydroisoquinoline alkaloids, which can be endogenous morphine precursors including norlaudanosoline (tetrahydropapaveroline [THP]), were regarded strictly as catecholamine-derived alkaloids found in brain tissues with weak binding activity for catecholamine receptors [154,160,161], suggesting another function. Ethanol treatment increased THP levels in L-DOPA treated rat brains [162]. Administration of ethanol and L-DOPA, as compared to the administration of L-DOPA only, markedly increased brain levels of dopamine as well. Thus, the dopamine-derived alkaloids, the tetrahydroisoquinolines (TIQs), were suspected of playing a role in the pathogenesis of alcoholism since alcohol induced the formation of THP and norcoclaurine in the rat brain [155]. The coupling of these processes was further strengthened in examining regional cerebral blood flow via PET scanning after consuming moderate amount of alcohol. It was found that neural structures were activated that were associated with the so-called cerebral reward system and the ascending reticular activating system [156].

The above observations become even more important since we now know animals can make morphine [21,163–172], including invertebrates [20,27,32,85,94,128,172–181], including human cancer cells [182–184]. Comparatively, in healthy *M. edulis,* morphine synthesis was demonstrated, including the use of reticuline, THP, DA and L-DOPA as precursors for making endogenous morphine [85,94,126–128]. THP was also found in mammalian tissues and invertebrate neural tissues. Taken together, it appears that ethanol increases

endogenous morphine levels and the levels of morphine's precursor's in neural tissues, suggesting that, in part, ethanol may exert its pleasure stimulating actions, as well as its addicting activities, via enhancing endogenous morphine,. It is highly significant that both nicotine and ethanol increase ganglionic morphine levels rapidly, providing a mechanism to initiate their pleasure and addicting actions.

COCAINE

Importantly, cocaine also exerts its mechanism of action via the alteration of dopaminergic processes, including those related to dopamine function [185]. In both invertebrates and mammals cocaine inhibits the ability to reuptake released dopamine via blocking its transporter, allowing more dopamine to be present for signaling [186,187]. We surmise that this dopamine may in time be channeled to the morphinergic system whereby morphine activity is enhanced. Furthermore, as recently demonstrated in invertebrate and human tissues cocaine promotes a statistically significant enhancement of ^{125}I-trace labeled morphine release [134]. The same occurs in human WBC [135], suggesting that cocaine's effect, in part, may occur via an enhancement of endogenous morphine signaling. Again, the physiological commonalties between nicotine, ethanol, and cocaine processing become obvious, linking these substances together, as they all neurophysiologically seem to converge, e.g., on morphine signaling.

REWARD, DOPAMINE, MORPHINE AND HEALTH

The brain's reward and motivation circuitry with its limbic components represents the crucial neurobiological system underlying pleasure phenomena. It not only serves pleasure and motivation, but also involves aspects of behavior, reproduction and sexual activity, emotion, belief and trust, memory, cognition, stress physiology and autonomic functions, relaxation and well-being – to name a few [5,10,12,39,188]. Neurotransmitters potentially acting on these CNS structures are, for example, dopamine, GABA, glutamate, serotonin, acetylcholine, morphine, nitric oxide, noradrenaline, cortisol, as well as endocannabinoids.

Natural rewards can be modulated by the activity of the brain's reward and motivation circuitry. Feeding, sexual activity or maternal behavior can be facilitated each by opiate activation of the reward system [29,38,54,70,74,189]. The origin of the VTA, i.e., ventral tegmental dopamine system seems to provide an important neurochemical interface where exogenous opiates and endogenous opioid peptides can activate a CNS mechanism involved in appetitive motivation and reward [1,5]. Mu and delta opioid receptors are involved in the reward mechanism of dopamine [69,70]. Obviously, endogenous morphinergic signaling also plays a role since endogenous morphine is a neurotransmitter in CNS [111]. This is substantiated by the demonstration of morphine release from nerve terminals [19,111,190,191]. Thus, dopamine and endogenous opiates/opioid peptides appear to be interconnected in the drug-pleasure-reward phenomenon, linking two signaling systems together. Additionally, endogenous morphine has been found in hippocampal tissues [171,192] and morphinergic signaling has been demonstrated to release constitutive nitric oxide here [193], linking morphine to limbic structures and nitric oxide effects. Thus, the VTA serves as a appetitive motivation system for diverse behaviors, since it controls both normal and pathological behaviors [1,5,24,78].

Artificial rewards and drugs, in contrast to natural stimuli that work, for example, by moderating sensory organ stimulation, are capable of acting directly on VTA and nucleus accumbens pathways, allowing only little flexibility and modulation to interfere (see above). Consequently, artificial rewards can diminish self-control and beneficial motivational behavior, leading to a potentially dangerous or detrimental outcome, i.e., motivational toxicity [1]. They may therefore be considered biologically senseless. Nonetheless, artificial and natural rewards cannot always be differentiated easily. The difference, however, could be a question of dose.

Because endogenous morphine is constitutively expressed and over-expressed following trauma, we have surmised that this opiate alkaloid normally down-regulates immune, gut, vascular and neural responsiveness on a basal level [56]. In addition, it also limits micro-environmental noise and post-inflammatory immune responsiveness [20,56,106,107,194]. Thus, it would not be surprising to find that alcohol, nicotine and cocaine share immune related pharmacology, e.g., down regulating, that may appear to be dysfunctional. This hypothesis extends the significance of this signaling beyond pleasure/reward processes. Simultaneously, it suggests that "reward/pleasure processes may have evolved as a motivational mechanism, ensuring that "relaxation"/recooperation may occur when appropriate further preserving an organisms health [29,30,189].

Taken together, natural rewarding activities and artificial chemical rewarding stimuli act at the same locations, but while natural activities are controlled by feedback mechanisms that activate aversive centers (i.e., aversive motivation), no such restrictions bind the responses to artificial stimuli [1,195]. Moreover, reward substrates that directly act on the brain's reward pathways are more potent than other rewards, such as food or water: subjects prefer to choose self-imposed starvation when forced to make a choice between obtaining food and water or direct electrical stimulation of the reward circuitry [196]. We can assume that nature has not made preparation, that is, has not planned for this artificial short-cut to occur.

The psychiatric implications of this system have been examined as well, including brain reward circuitry [5,110,111,197]. These data contribute to an evolving hypothesis linking the reinforcing and addictive properties of a variety of drugs of abuse to convergent mechanisms involving endogenous morphine signaling and establish an opiate foundation as a unifying principle by which we may advance our understanding of polymodal drug abuse mechanisms. It even transcends these studies by firmly placing this system within the realm of mental health as well.

CONCLUSIONS

Pleasure can serve health, but is also capable of promoting addiction and other dangerous outcomes or behaviors (i.e., motivational toxicity). It is a complex neurobiological phenomenon, relying on reward circuitry activity and limbic processes. Artificial stimulants (e.g., addictive drugs)

or 'too much' of a pleasurable activity may not be as beneficial, since flexibility and natural control of behaviors may be deteriorated. Clearly, addiction includes a loss of control over normal behaviors and appetitive motivational goals. Addictive drugs, in addition, are capable of directly and strongly acting on reward pathways, i.e., dopamine and morphine signaling, thereby influencing motivation physiology. Importantly, these novel insights into the pleasure phenomena also provide insights into mental health, namely, via morphinergic signaling, which occurs in critical areas of the brain involved with cognition.

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REFERENCES:

1. Bozarth MA: Pleasure systems in the brain. In: Wartburton DM, editors. Pleasure: The politics and the reality. New York, Wiley & Sons, 1994
2. Nestler EJ, Malenka RC: The addicted brain. Sci Am, 2004; 290: 78–85
3. Cannon CM, Bseikri MR: Is dopamine required for natural reward? Physiol Behav, 2004; 81: 741–48
4. Salamone JD: Will the last person who uses the term 'reward' please turn out the lights? Comments on processes related to reinforcement, learning, motivation and effort. Addict Biol, 2006; 11: 43–44
5. Esch T, Guarna M, Bianchi E et al: Commonalities in the central nervous system's involvement with complementary medical therapies: Limbic morphinergic processes. Med Sci Monit, 2004; 10(6): MS6–17
6. Esch T, Stefano GB: The neurobiology of pleasure, reward processes, addiction and their health implications. Neuroendocrinology Letters, 2004; 25: 235–51
7. Kream RM, Stefano GB: Morphine synthesis in animals. Med Sci Monit, 2006; 12(10): ED1–2
8. Zhu W, Pryor SC, Putnam J et al: Opiate alkaloids and nitric oxide production in the nematode Ascaris suum. J Parasitol, 2004; 90: 15–22
9. Salzet M, Stefano GB: The endocannabinoid system in invertebrates. Prostaglandins Leukotrienes & Essential Fatty Acids, 2002; 66: 353–61
10. Stefano GB, Fricchione GL, Slingsby BT, Benson H: The placebo effect and relaxation response: Neural processes and their coupling to constitutive nitric oxide. Brain Res Rev, 2001; 35: 1–19
11. Hui KKS, Liu J, Makris N et al: Acupuncture modulates the limbic system and subcortical gray structures of the human brain: Evidence from fMRI studies in normal subjects. Hum Brain Mapp, 2000; 9: 13–25
12. Esch T, Stefano GB, Fricchione GL, Benson H: The role of stress in neurodegenerative diseases and mental disorders. Neuroendocrinology Letters, 2002; 23: 199–208
13. Davidson RJ, Irwin W: The functional neuroanatomy of emotion and affective style. Trends Cogn Sci, 1999; 3: 11–21
14. Esch T: [Music medicine: Music in association with harm and healing]. Musikphysiol Musikermed, 2003; 10: 213–24
15. Nestler EJ: Molecular basis of long-term plasticity underlying addiction. Nat Rev Neurosci, 2001; 2: 119–28
16. Nestler EJ, Malenka RC, Hyman SE: Molecular basis of neuropharmacology. Columbus, McGraw-Hill, 2001
17. Stefano GB, Fricchione GL: The biology of deception: Emotion and morphine. Med Hypotheses, 1995; 49: 51–54
18. Bianchi E, Guarna M, Tagliamonte A: Immunocytochemical localization of endogenous codeine and morphine. Adv Neuroimmunol, 1994; 4: 83–92
19. Guarna M, Bianchi E, Bartolini A et al: Anti-mu opioid antiserum against the third external loop of the cloned mu opioid receptor acts as a mu receptor neutral antagonist. 2002
20. Stefano GB, Goumon Y, Casares F et al: Endogenous morphine. Trends Neurosci, 2000; 9: 436–42
21. Zhu W, Ma Y, Bell A et al: Presence of morphine in rat amygdala: Evidence for the mu3 opiate receptor subtype via nitric oxide release in limbic structures. Med Sci Monit, 2004; 10(12): BR433–39
22. Olds J: Drives and reinforcements: Behavioral studies of hypothalamic functions. New York, Raven Press, 1977
23. Wise RA: Catecholamine theories of reward: A critical review. Brain Res, 1978; 152: 215–47
24. Bozarth MA: Ventral tegmental reward system. In: Oreland L and Engel J (eds.) Brain reward systems and abuse. New York, Raven Press, 1987
25. Rodriguez dF, Navarro M: Role of the limbic system in dependence on drugs. Ann Med, 1998; 30: 397–405
26. Weiss F, Koob GF: Drug addiction: Functional neurotoxicity of the brain reward systems. Neurotox Res, 2001; 3: 145–56
27. Zhu W, Mantione KJ, Shen L et al: Norlaudanosoline and nicotine increase endogenous ganglionic morphine levels: Nicotine addiction. Cell Mol Neurobiol, 2006; 26: 1037–45
28. Troland LT: The fundamentals of human motivation. New York, Van Nostrand Reinhold, 1928
29. Esch T, Kim JW, Stefano GB: Neurobiological implications of eating healthy. Neuro Endocrinol Lett, 2006; 27
30. Salamon E, Esch T, Stefano GB: Pain and relaxation (review). Int J Mol Med, 2006; 18: 465–70
31. White NM, Milner PM: The psychobiology of reinforcers. Annu Rev Psychol, 1992; 43: 443–71
32. Stefano GB, Fricchione GL, Goumon Y, Esch T: Pain, immunity, opiate and opioid compounds and health. Med Sci Monit, 2005; 11(5): MS47–53
33. Everitt BJ, Robbins TW: Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. Nat Neurosci, 2005; 8: 1481–89
34. Salamone JD, Correa M: Motivational views of reinforcement: implications for understanding the behavioral functions of nucleus accumbens dopamine. Behav Brain Res, 2002; 137: 3–25
35. Sanchis-Segura C, Spanagel R: Behavioural assessment of drug reinforcement and addictive features in rodents: an overview. Addict Biol, 2006; 11: 2–38
36. Berridge KC: Pleasures of the brain. Brain Cogn, 2003; 52: 106–28
37. Goldstein RZ, Volkow ND: Drug addiction and its underlying neurobiological basis: neuroimaging evidence for the involvement of the frontal cortex. Am J Psychiatry, 2002; 159: 1642–52
38. Mitchell JB, Stewart J: Facilitation of sexual behaviors in the male rat associated with intra-VTA injections of opiates. Pharmacol Biochem Behav, 1990; 35: 643–50
39. Esch T, Fricchione GL, Stefano GB: The therapeutic use of the relaxation response in stress-related diseases. Med Sci Monit, 2003; 9(2): RA23–34
40. Stefano GB, Esch T, Cadet P et al: Endocannabinoids as autoregulatory signaling molecules: Coupling to nitric oxide and a possible association with the relaxation response. Med Sci Monit, 2003; 9(4): RA63–75
41. Aung AT, Hickman NJ III, Moolchan ET: Health and performance related reasons for wanting to quit: Gender differences among teen smokers. Subst Use Misuse, 2003; 38: 1095–107
42. Botvin GJ, Griffin KW, Diaz T et al: Smoking initiation and escalation in early adolescent girls: One-year follow-up of a school-based prevention intervention for minority youth. J Am Med Womens Assoc, 1999; 54: 139–43, 152
43. Cachelin FM, Weiss JW, Garbanati JA: Dieting and its relationship to smoking, acculturation, and family environment in Asian and Hispanic adolescents. Eating Disorders: The Journal of Treatment and Prevention, 2003; 11: 51–61
44. Dzien A, Dzien-Bischinger C, Hoppichler F, Lechleitner M: The metabolic syndrome as a link between smoking and cardiovascular disease. Diabetes Obes Metab, 2004; 6: 127–32
45. Epstein JA, Botvin GJ, Spoth R: Predicting smoking among rural adolescents: Social and cognitive processes. Nicotine Tob Res, 2003; 5: 485–91
46. Gilman SE, Abrams DB, Buka SL: Socioeconomic status over the life course and stages of cigarette use: Initiation, regular use, and cessation. J Epidemiol Community Health, 2003; 57: 802–8
47. Jefferis B, Graham H, Manor O, Power C: Cigarette consumption and socio-economic circumstances in adolescence as predictors of adult smoking. Addiction, 2003; 98: 1765–72
48. Stangl V, Baumann G, Stangl K: Coronary atherogenic risk factors in women. Eur Heart J, 2002; 23: 1738–52
49. Yorulmaz F, Akturk Z, Dagdeviren N, Dalkilic A: Smoking among adolescents: Relation to school success, socioeconomic status nutrition and self-esteem. Swiss Med Wkly, 2002; 132: 449–54

50. Renaud SC, Beswick AD, Fehily AM et al: Alcohol and platelet aggregation: the Caerphilly Prospective Heart Disease Study. Am J Clin Nutr, 1992; 55: 1012–17
51. Takkouche B, Regueira-Mendez C, Garcia-Closas R et al: Intake of wine, beer, and spirits and the risk of clinical common cold. Am J Epidemiol, 2002; 155: 853–58
52. Truelsen T, Thudium D, Gronbaek M: Amount and type of alcohol and risk of dementia: the Copenhagen City Heart Study. Neurology, 2002; 59: 1313–19
53. Hoidrup S, Gronbaek M, Gottschau A et al: Alcohol intake, beverage preference, and risk of hip fracture in men and women. Copenhagen Centre for Prospective Population Studies. Am J Epidemiol, 1999; 149: 993–1001
54. Hamilton ME, Bozarth MA: Feeding elicited by dynorphin (1-13) microinjections into the ventral tegmental area in rats. Life Sci, 1988; 43: 941–46
55. Heath RG: Pleasure response of human subjects to direct stimulation of the brain. In: Heath RG (eds.) The Role of Pleasure in Human Behavior. New York, Hoeber, 1964
56. Stefano GB, Scharrer B: Endogenous morphine and related opiates, a new class of chemical messengers. Adv Neuroimmunol, 1994; 4: 57–68
57. Stefano GB, Scharrer B, Bilfinger TV et al: A novel view of opiate tolerance. Adv Neuroimmunol, 1996; 6: 265–77
58. Robinson TE, Berridge KC: Incentive-sensitization and addiction. Addiction, 2001; 96: 103–14
59. de la Fuente-Fernandez R, Schulzer M, Stoessl AJ: The placebo effect in neurological disorders. Lancet Neurol, 2002; 1: 85–91
60. McClung CA, Nestler EJ: Regulation of gene expression and cocaine reward by CREB and DeltaFosB. Nat Neurosci, 2003; 6: 1208–15
61. Zhang L, Lou D, Jiao H et al: Cocaine-induced intracellular signaling and gene expression are oppositely regulated by the dopamine D1 and D3 receptors. J Neurosci, 2004; 24: 3344–54
62. Hutcheson DM, Everitt BJ, Robbins TW, Dickinson A: The role of withdrawal in heroin addiction: enhances reward or promotes avoidance? Nat Neurosci, 2001; 4: 943–47
63. Ahmed SH: Imbalance between drug and non-drug reward availability: a major risk factor for addiction. Eur J Pharmacol, 2005; 526: 9–20
64. Vanderschuren LJ, Everitt BJ: Behavioral and neural mechanisms of compulsive drug seeking. Eur J Pharmacol, 2005; 526: 77–88
65. Murphy CA, Russig H, Pezze MA et al: Amphetamine withdrawal modulates FosB expression in mesolimbic dopaminergic target nuclei: effects of different schedules of administration. Neuropharmacology, 2003; 44: 926–39
66. Nylander I, Vlaskovska M, Terenius L: The effects of morphine treatment and morphine withdrawal on the dynorphin and enkephalin systems in Sprague-Dawley rats. Psychopharmacology (Berl), 1995; 118: 391–400
67. Skoubis PD, Lam HA, Shoblock J et al: Endogenous enkephalins, not endorphins, modulate basal hedonic state in mice. Eur J Neurosci, 2005; 21: 1379–84
68. Green TA, Alibhai IN, Hommel JD et al: Induction of inducible cAMP early repressor expression in nucleus accumbens by stress or amphetamine increases behavioral responses to emotional stimuli. J Neurosci, 2006; 26: 8235–42
69. Kornetsky C: Brain-stimulation reward, morphine-induced oral stereotypy, and sensitization: implications for abuse. Neurosci Biobehav Rev, 2004; 27: 777–86
70. Thompson AC, Kristal MB: Opioids in the ventral tegmental area facilitate the onset of maternal behavior in the rat. Society for Neuroscience Abstracts, 1994; 18: 659
71. de la Fuente-Fernandez R, Ruth TJ, Sossi V et al: Expectation and dopamine release: mechanism of the placebo effect in Parkinson's disease. Science, 2001; 293: 1164–66
72. Spanagel R, Weiss F: The dopamine hypothesis of reward: past and current status. Trends Neurosci, 1999; 22: 521–27
73. Sapolsky RM: Stress and plasticity in the limbic system. Neurochem Res, 2003; 28: 1735–42
74. Esch T, Stefano GB: The Neurobiology of Love. Neuroendocrinology Letters, 2005; 26: 175–92
75. Summers CH, Korzan WJ, Lukkes JL et al: Does serotonin influence aggression? comparing regional activity before and during social interaction. Physiol Biochem Zool, 2005; 78: 679–94
76. Morgan D, Grant KA, Gage HD et al: Social dominance in monkeys: dopamine D2 receptors and cocaine self-administration. Nat Neurosci, 2002; 5: 169–74
77. Morgan D, Grant KA, Prioleau OA et al: Predictors of social status in cynomolgus monkeys (Macaca fascicularis) after group formation. Am J Primatol, 2000; 52: 115–31
78. Bozarth MA: The mesolimbic dopamine system as a model reward system. In: Willner P and Scheel-Krüger J (eds.) The Mesolimbic Dopamine System: From Motivation to Action. London, Wiley & Sons, 1991
79. Fibiger HC, Phillips AG: Dopamine and the neural mechanisms of reinforcement. In: Horn AS, Westerink BHC, Korf J (eds.) The Neurobiology of Dopamine. New York, Academic Press, 1979; 597–615
80. Esch T, Gesenhues S: [Terminology, classification and rational diagnostics in depression]. Psycho, 2002; 28: S21–27
81. Bozarth MA: New perspectives on cocaine addiction: Recent findings from animal research. Can J Physiol Pharmacol, 1989; 67: 1158–67
82. Rossetti ZL, Hmaidan Y, Gessa GL: Marked inhibition of mesolimbic dopamine release: A common feature of ethanol, morphine, cocaine and amphetamine abstinence in rats. Eur J Pharmacol, 1992; 221: 227–34
83. Wise RA, Bozarth MA: A psychomotor stimulant theory of addiction. Psychol Rev, 1987; 94: 469–92
84. Bozarth MA: Drug addiction as a psychobiological process. In: Wartburton DM, editors. Addiction Controversies. London: Harwood Academic Publishers, 1990
85. Zhu W, Mantione KJ, Shen L et al: Tyrosine and tyramine increase endogenous ganglionic morphine and dopamine levels *in vitro* and *in vivo*: CYP2D6 and tyrosine hydroxylase modulation demonstrates a dopamine coupling. Med Sci Monit, 2005; 11(11): BR397–404
86. Zhu W, Cadet P, Baggerman G et al: Human white blood cells synthesize morphine: CYP2D6 modulation. J Imumnol, 2005; 175: 7357–62
87. Kream RM, Stefano GB: *De novo* biosynthesis of morphine in animal cells: An evidence-based model. Med Sci Monit, 2006; 12(10): RA207–19
88. Pierce RC, Kumaresan V: The mesolimbic dopamine system: The final common pathway for the reinforcing effect of drugs of abuse? Neurosci Biobehav Rev, 2006; 30: 215–38
89. Larsson A, Engel JA: Neurochemical and behavioral studies on ethanol and nicotine interactions. Neurosci Biobehav Rev, 2004; 27: 713–20
90. Dohrman DP, Reiter CK: Chronic ethanol reduces nicotine-induced dopamine release in PC12 cells. Alcohol Clin Exp Res, 2003; 27: 1846–51
91. Larsson A, Svensson L, Soderpalm B, Engel JA: Role of different nicotinic acetylcholine receptors in mediating behavioral and neurochemical effects of ethanol in mice. Alcohol, 2002; 28: 157–67
92. Tizabi Y, Copeland RL Jr, Louis VA, Taylor RE: Effects of combined systemic alcohol and central nicotine administration into ventral tegmental area on dopamine release in the nucleus accumbens. Alcohol Clin Exp Res, 2002; 26: 394–99
93. Soderpalm B, Ericson M, Olausson P et al: Nicotinic mechanisms involved in the dopamine activating and reinforcing properties of ethanol. Behav Brain Res, 2000; 113: 85–96
94. Zhu W, Ma Y, Cadet P et al: Presence of reticuline in rat brain: A pathway for morphine biosynthesis. Mol Brain Res, 2003; 117: 83–90
95. Pai HV, Kommaddi RP, Chinta SJ et al: A frameshift mutation and alternate splicing in human brain generate a functional form of the pseudogene cytochrome P4502D7 that demethylates codeine to morphine. J Biol Chem, 2004; 279: 27383–89
96. Gasche Y, Daali Y, Fathi M et al: Codeine intoxication associated with ultrarapid CYP2D6 metabolism. N Engl J Med, 2004; 351: 2827–31
97. Amann T, Roos PH, Huh H, Zenk MH: Purification and characterization of a cytochrome P450 enzyme from pig liver, catalyzing the phenol oxidative coupling of (R)-reticuline to salutaridine, the critical step in morphine biosynthesis. Heterocycles, 1995; 40: 425–40
98. Funae Y, Kishimoto W, Cho T et al: CYP2D in the brain. Drug Metab Pharmacokinet, 2003; 18: 337–49
99. Hiroi T, Imaoka S, Funae Y: Dopamine formation from tyramine by CYP2D6. Biochem Biophys Res Commun, 1998; 249: 838–43
100. Lind AB, Wadelius M, Darj E et al: Gene expression of cytochrome P450 1B1 and 2D6 in leukocytes in human pregnancy. Pharmacol Toxicol, 2003; 92: 295–99
101. Touw DJ: Clinical implications of genetic polymorphisms and drug interactions mediated by cytochrome P-450 enzymes. Drug Metabol Drug Interact, 1997; 14: 55–82
102. Stefano GB, Zhao X, Bailey D et al: High affinity dopamine binding to mouse thymocytes and Mytilus edulis (Bivalvia) hemocytes. J Neuroimmunol, 1989; 21: 67–74

103. Cosentino M, Marino F, Bombelli R et al: Endogenous catecholamine synthesis, metabolism, storage and uptake in human neutrophils. Life Sci, 1999; 64: 975–81
104. Bergquist J, Silberring J: Identification of catecholamines in the immune system by electrospray ionization mass spectrometry. Rapid Commun Mass Spectrom, 1998; 12: 683–88
105. Basu S, Dasgupta PS: Dopamine, a neurotransmitter, influences the immune system. J Neuroimmunol, 2000; 102: 113–24
106. Stefano GB, Scharrer B, Smith EM et al: Opioid and opiate immunoregulatory processes. Crit Rev in Immunol, 1996; 16: 109–44
107. Stefano GB: Autoimmunovascular regulation: Morphine and anandamide stimulated nitric oxide release. J Neuroimmunol, 1998; 83: 70–76
108. Saurer TB, Carrigan KA, Ijames SG, Lysle DT: Morphine-induced alterations of immune status are blocked by the dopamine D2-like receptor agonist 7-OH-DPAT. J Neuroimmunol, 2004; 148: 54–62
109. Dani JA, Harris RA: Nicotine addiction and comorbidity with alcohol abuse and mental illness. Nat Neurosci, 2005; 8: 1465–70
110. Fricchione GL, Stefano GB: Placebo neural systems: Nitric oxide, morphine and the dopamine brain reward and motivation circuitries. Med Sci Monit, 2005; 11(5): MS54–65
111. Guarna M, Ghelardini C, Galeotti N et al: Neurotransmitter role of endogenous morphine in CNS. Med Sci Monit, 2005; 11(6): RA190–93
112. Pryor SC, Zhu W, Cadet P et al: Endogenous morphine: opening new doors for the treatment of pain and addiction. Expert Opin Biol Ther, 2005; 5: 893–906
113. de Haan L, Booij J, Lavalaye J et al: Occupancy of dopamine D2 receptors by antipsychotic drugs is related to nicotine addiction in young patients with schizophrenia. Psychopharmacology (Berl), 2006; 183: 500–5
114. Nakajima M, Yokoi T: Interindividual variability in nicotine metabolism: C-oxidation and glucuronidation. Drug Metab Pharmacokinet, 2005; 20: 227–35
115. Biala G, Weglinska B: On the mechanism of cross-tolerance between morphine- and nicotine-induced antinociception: Involvement of calcium channels. Prog Neuropsychopharmacol Biol Psychiatry, 2005
116. Biala G, Weglinska B: Calcium channel antagonists attenuate cross-sensitization to the rewarding and/or locomotor effects of nicotine, morphine and MK-801. J Pharm Pharmacol, 2004; 56: 1021–28
117. Grinevich VP, Crooks PA, Sumithran SP et al: N-n-alkylpyridinium analogs, a novel class of nicotinic receptor antagonists: selective inhibition of nicotine-evoked [3H] dopamine overflow from superfused rat striatal slices. J Pharmacol Exp Ther, 2003; 306: 1011–20
118. Brody AL, Mandelkern MA, Olmstead RE et al: Gene variants of brain dopamine pathways and smoking-induced dopamine release in the ventral caudate/nucleus accumbens. Arch Gen Psychiatry, 2006; 63: 808–16
119. Geng Y, Savage SM, Johnson LJ et al: Effects of nicotine on the immune response. I. Chronic exposure to nicotine impairs antigen receptor-mediated signal transduction in lymphocytes. Tox & Applied Pharm, 1995; 135: 268–78
120. Geng Y, Savage SM, Razani-Boroujerdi S, Sopori ML: Effects of nicotine on the immune response. II. Chronic nicotine treatment induces T cell anergy. J Immunol, 1996; 156: 2384–90
121. Savage SM, Donaldson LA, Cherian S et al: Effects of cigarette smoke on the immune response. II. Chronic exposure to cigarette smoke inhibits surface immunoglobulin- mediated responses in B cells. Tox & Applied Pharm, 1991; 111: 523–29
122. Sopori ML, Gairola CG, DeLucia AJ et al: Immune responsiveness of monkeys exposed chronically to cigarette smoke. Clin Immunol Immunopathol, 1985; 36: 338–44
123. Sopori ML, Cherian S, Chilukuri R, Shopp GM: Cigarette smoke causes inhibition of the immune response to intratracheally administered antigens. Tox & Applied Pharm, 1989; 97: 489–99
124. Sopori ML, Savage SM, Christner RF et al: Cigarette smoke and the immune response: mechanism of nicotine – induced immunosuppression. Adv Biosci, 1993; 86: 663–72
125. Sopori ML, Goud NS, Kaplan AM: Effects of tobacco smoke on the immune system. In: Dean JH, Luster MI, Munson AE et al: (eds.) Immunotoxicology and immunopharmacology. New York, Raven Press, 1994; 413–34
126. Zhu W, Stefano GB: Reticuline exposure to invertebrate ganglia increases endogenous morphine levels. Neuro Endocrinol Lett, 2004; 25: 323–30
127. Zhu W, Mantione KJ, Shen L, Stefano GB: *In vivo* and *in vitro* L-DOPA exposure increases ganglionic morphine levels. Med Sci Moni, 2005; 11(5): MS1–5
128. Zhu W, Ma Y, Stefano GB: Presence of isoquinoline alkaloids in molluscan ganglia. Neuroendocrinol Lett, 2002; 23: 329–34
129. Wright TJ, Huddart H: The nature of the acetylcholine and 5-hydroxytryptamine receptors in buccal smooth muscle of the pest slug Deroceras reticulatum. J Comp Physiol [B], 2002; 172: 237–49
130. Dowell C, Olivera BM, Garrett JE et al: Alpha-conotoxin PIA is selective for alpha6 subunit-containing nicotinic acetylcholine receptors. J Neurosci, 2003; 23: 8445–52
131. Lee CM, Lin JT, Hwang JC: Pharmacological properties of ACh receptors on the heart of the marine bivalve Meretrix lusoria. Chin J Physiol, 1998; 41: 19–24
132. Vehovszky A, Salanki J: Pharmacological characterization of postsynaptic potentials evoked in the bimodal pacemaker neuron of Helix pomatia L. Acta Physiol Hung, 1983; 62: 35–46
133. Hellwig G, Achazi RK: ACh and 5-HT induced changes in the concentration of cytosolic inositol trisphosphate (InsP3) and inositol bisphosphate (InsP2) in the ABRM of Mytilus edulis L. Comp Biochem Physiol C, 1991; 100: 343–48
134. Zhu W, Mantione KJ, Casares FM et al: Alcohol-, nicotine-, and cocaine-evoked release of morphine from invertebrate ganglia: Model system for screening drugs of abuse. Med Sci Monit, 2006; 12(5): BR155–61
135. Zhu W, Mantione K, Kream RM, Stefano GB: Alcohol-, Nicotine-, and Cocaine-Evoked Release of Morphine from Human White Blood Cells: Substances of Abuse Actions Converge on Endogenous Morphine Release. Med Sci Monit, 2006; 12(11): BR350–54
136. Zhu W, Mantione KJ, Casares FM et al: Cholinergic regulation of endogenous morphine release from lobster nerve cord. Med Sci Monit, 2006; 12(9): BR295–301
137. Lummis SC, Sattelle DB: Binding of N-[propionyl-3H]propionylated alpha-bungarotoxin and L-[benzilic-4,4'-3H] quinuclidinyl benzilate to CNS extracts of the cockroach Periplaneta americana. Comp Biochem Physiol C, 1985; 80: 75–83
138. Zayas RM, Qazi S, Morton DB, Trimmer BA: Nicotinic-acetylcholine receptors are functionally coupled to the nitric oxide/cGMP-pathway in insect neurons. J Neurochem, 2002; 83: 421–31
139. Jumblatt JE, Marquis JK, Mautner HG: On the specificity of 125-I-alpha-bungarotoxin binding to axonal membranes. J Neurochem, 1981; 37: 392–400
140. Fossier P, Poulain B, Baux G, Tauc L: Both presynaptic nicotinic-like and muscarinic-like autoreceptors regulate acetylcholine release at an identified neuro-neuronal synapse of Aplysia. Pflugers Arch, 1988; 411: 345–52
141. Fu WM, Liou HC, Chen YH: Nerve terminal currents induced by autoreception of acetylcholine release. J Neurosci, 1998; 18: 9954–61
142. Wilson DF, West AE, Lin Y: Inhibitory action of nicotinic antagonists on transmitter release at the neuromuscular junction of the rat. Neurosci Lett, 1995; 186: 29–32
143. Wilson DF, Thomsen RH: Effects of hexamethonium on transmitter release from the rat phrenic nerve. Neurosci Lett, 1992; 143: 79–82
144. Woods AS, Moyer SC, Wang HY, Wise RA: Interaction of chlorisondamine with the neuronal nicotinic acetylcholine receptor. J Proteome Res, 2003; 2: 207–12
145. Fisher M, Huangfu D, Shen TY, Guyenet PG: Epibatidine, an alkaloid from the poison frog Epipedobates tricolor, is a powerful ganglionic depolarizing agent. J Pharmacol Exp Ther, 1994; 270: 702–7
146. Bannon AW, Gunther KL, Decker MW: Is epibatidine really analgesic? Dissociation of the activity, temperature, and analgesic effects of (±)-epibatidine. Pharmacol Biochem Behav, 1995; 51: 693–98
147. Yogeeswari P, Sriram D, Ratan BT, Thirumurugan R: Epibatidine and its analogues as nicotinic acetylcholine receptor agonist: an update. Nat Prod Res, 2006; 20: 497–505
148. Nieto-Fernandez FE, Mattocks DW, Cavani F et al: Morphine coupling to invertebrate immunocyte nitric oxide release is dependent on intracellular calcium transients. Comp Biochem Physiol, 1999; 123: 295–99
149. Catapane EJ, Stefano GB, Aiello E: Pharmacological study of the reciprocal dual innervation of the lateral ciliated gill epithelium by the CNS of Mytilus edulis. J Exp Biol, 1978; 74: 101–13
150. Catapane EJ, Stefano GB, Aiello E: Neurophysiological correlates of the dopaminergic Cilio-inhibitory mechanism. J Exp Biol, 1979; 83: 315–23

151. Stefano GB, Catapane EJ, Stefano JM: Temperature dependent ciliary rhythmicity in Mytilus edulis and the effects of monoaminergic agents on its manifestation. Biol Bull, 1977; 153: 618–29

152. Aiello E, Hager E, Akiwumi C, Stefano GB: An opioid mechanism modulates central and not peripheral dopaminergic control of ciliary activity in the marine mussel Mytilus edulis. Cell Mol Neurobiol, 1986; 6: 17–30

153. Stefano GB, Catapane EJ, Aiello E: Dopaminergic agents: Influence on serotonin in the molluscan nervous system. Science, 1976; 194: 539–41

154. Davis VE, Walsh MJ: Alcohol, amines and alkaloids: a possible biochemical basis for alcohol addiction. Science, 1970; 167: 1005–7

155. Haber H, Roske I, Rottmann M et al: Alcohol induces formation of morphine precursors in the striatum of rats. Life Sciences, 1997; 60: 79–89

156. Ingvar M, Ghatan PH, Wirsen-Meurling A et al: Alcohol activates the cerebral reward system in man. J Stud Alcohol, 1998; 59: 258–69

157. Kreek MJ: Opioid interactions with alcohol. Adv Alcohol Subst Abuse, 1984; 3: 35–46

158. Rada P, Johnson DF, Lewis MJ, Hoebel BG: In alcohol-treated rats, naloxone decreases extracellular dopamine and increases acetylcholine in the nucleus accumbens: evidence of opioid withdrawal. Pharmacol Biochem Behav, 2004; 79: 599–605

159. DiChiara G, Acquas E, Carboni E: Dopamine and drug-induced motivation. In: Gessa GL, Serra G (eds.) Dopamine and Mental Depression. Oxford, Pergamon Press, 1990; 27–38

160. Nimit Y, Schulze I, Cashaw JL et al: Interaction of catecholamine-derived alkaloids with central neurotransmitter receptors. J Neurosci Res, 1983; 10: 175–89

161. Seevers MH, Davis VE, Walsh MJ: Morphine and ethanol physical dependence: a critique of a hypothesis. Science, 1970; 170: 1113–15

162. Cashaw JL, Geraghty CA, McLaughlin BR, Davis VE: Effect of acute ethanol administration on brain levels of tetrahydropapaveroline in L-dopa-treated rats. J Neurosci Res, 1987; 18: 497–503

163. Cardinale GJ, Donnerer J, Finck AD et al: Morphine and codeine are endogenous components of human cerebrospinal fluid. Life Sci, 1987; 40: 301–6

164. Donnerer J, Oka K, Brossi A et al: Presence and formation of codeine and morphine in the rat. Proc Natl Acad Sci USA, 1986; 83: 4566–67

165. Donnerer J, Cardinale G, Coffey J et al: Chemical characterization and regulation of endogenous morphine and codeine in the rat. J Pharmacol Exp Ther, 1987; 242: 583–87

166. Epple A, Navarro I, Horak P, Spector S: Endogenous morphine and codeine: release by the chromaffin cells of the eel. Life Sci, 1993; 52: PL117–21

167. Gintzler AR, Levy A, Spector S: Antibodies as a means of isolating and characterizing biologically active substances: Presence of a non-peptide morphine-like compound in the central nervous system. Proc Natl Acad Sci USA, 1976; 73: 2132–36

168. Kodaira H, Spector S: Transformation of thebaine to oripavine, codeine, and morphine by rat liver,kidney, and brain microsomes. Proc Natl Acad Sci USA, 1988; 85: 1267–71

169. Kodaira H, Listek CA, Jardine I et al: Identification of the convusant opiate thebaine in the mammalian brain. Proc Natl Acad Sci USA, 1989; 86: 716–19

170. Spector S, Kantrowitz JD, Oka K: Presence of endogenous morphine in toad skin. Prog Clin Biol Res, 1985; 192: 329–32

171. Spector S, Munjal I, Schmidt DE: Endogenous morphine and codeine. Possible role as endogenous anticonvulsants. Brain Res, 2001; 915: 155–60

172. Stefano GB, Digenis A, Spector S et al: Opiate-like substances in an invertebrate, an opiate receptor on invertebrate and human immunocytes, and a role in immunosuppression. Proc Natl Acad Sci USA, 1993; 90: 11099–103

173. Leung MK, Dissous C, Capron A et al: Schistosoma mansoni: The presence and potential use of opiate-like substances. Exp Parasit, 1995; 81: 208–15

174. Stefano GB, Leung MK, Bilfinger TV, Scharrer B: Effect of prolonged exposure to morphine on responsiveness of human and invertebrate immunocytes to stimulatory molecules. J Neuroimmunol, 1995; 63: 175–81

175. Allen S, Khan S, Al-Mohanna F et al: Native low density lipoprotein-induced calcium transients trigger VCAM-1 and E-selectin expression in cultured human vascular endothelial cells. J Clin Invest, 1998; 101: 1064–75

176. Zhu W, Cadet P, Mantione KJ et al: Response to Comment on „Human White Blood Cells Synthesize Morphine: CYP2D6 Modulation". J Immunol, 2006; 176: 5704

177. Goumon Y, Casares F, Zhu W, Stefano GB: The presence of morphine in ganglionic tissues of Modiolus deminissus: A highly sensitive method of quantitation for morphine and its derivatives. Mol Brain Res, 2001; 86: 184–88

178. Goumon Y, Casares F, Pryor S et al: Ascaris suum, an internal parasite, produces morphine. J Immunol, 2000; 165: 339–43

179. Stefano GB, Cadet P, Rialas CM et al: Invertebrate Opiate Immune and Neural Signaling. In: Machelska H, Stein C (eds.) Immune Mechanisms of Pain and Analgesia. New York, NY, Plenum Publ., 2003; 126–47

180. Belkowski SM, Zhu J, Liu-Chen LY et al: Detection of kappa-opioid receptor mRNA in immature T cells. Adv Exp Med Biol, 1995; 373: 11–16

181. Zhu W, Baggerman G, Secor WE et al: Dracunculus medinensis and Schistosoma mansoni contain opiate alkaloids. Ann Trop Med Parasitol, 2002; 96: 309–16

182. Boettcher C, Fellermeier M, Boettcher C et al: How human neuroblastoma cells make morphine. Proc Natl Acad Sci USA, 2005; 102: 8495–500

183. Poeaknapo C, Schmidt J, Brandsch M et al: Endogenous formation of morphine in human cells. Proc Natl Acad Sci USA, 2004; 101: 14091–96

184. Zhu W, Baggerman G, Goumon Y et al: Identification of morphine and morphine-6-glucuronide in the adrenal medullary chromaffin PC-12 cell line by nano electrospray ionization double quadrupole orthogonal acceleration time of flight mass spectrometry. Eur J of Mass Spect, 2001; 7: 25–28

185. Stefano GB, Braham E, Sinisterra JI et al: The effects of cocaine on peripheral monoaminergic regulatory mechanisms in the gill of Mytilus edulis. Symposia Biologica Hungarica, 1988; 36: 51–62

186. Wu X, Gu HH: Cocaine affinity decreased by mutations of aromatic residue phenylalanine 105 in the transmembrane domain 2 of dopamine transporter. Mol Pharmacol, 2003; 63: 653–58

187. Jayanthi LD, Apparsundaram S, Malone MD et al: The Caenorhabditis elegans gene T23G5.5 encodes an antidepressant- and cocaine-sensitive dopamine transporter. Mol Pharmacol, 1998; 54: 601–9

188. Altenmuller EO: [Apollo in us: How the brain processes music]. Musikphysiol Musikermed, 2002; 9: 24

189. Stefano GB, Fricchione GL, Esch T: Relaxation: Molecular and physiological significance. Med Sci Monit, 2006; 12(9): HY21–31

190. Guarna M, Neri C, Petrioli F, Bianchi E: Potassium-induced release of endogenous morphine form rat brain slices. J Neurochem, 1998; 70: 147–52

191. Guarna M, Bianchi E, Bartolini A et al: Endogenous morphine modulates acute thermonociception in mice. J Neurochem, 2002; 80: 271–77

192. Bianchi E, Alessandrini C, Guarna M, Tagliamonte A: Endogenous codeine and morphine are stored in specific brain neurons. Brain Res, 1993; 627: 210–15

193. de la Torre JC, Pappas BA, Prevot V et al: Hippocampal nitric oxide upregulation precedes memory loss and A beta I-40 accumulation after chronic brain hypoperfusion in rats. Neurological Research, 2003; 25: 635–41

194. Stefano GB, Goumon Y, Bilfinger TV et al Basal nitric oxide limits immune, nervous and cardiovascular excitation: Human endothelia express a mu opiate receptor. Prog Neurobiol, 2000; 60: 513–30

195. Vetulani J: Drug addiction. Part II. Neurobiology of addiction. Pol J Pharmacol, 2001; 53: 303–17

196. Routtenberg A, Lindy J: Effects of the availability of rewarding septal and hypothalamic stimulation on bar pressing for food under conditions of deprivation. J Comp Physiol Psychol, 1965; 60: 158–61

197. Fricchione GL, Mendoza A, Stefano GB: Morphine and its psychiatric implications. Adv Neuroimmunol, 1994; 4: 117–32