Opioids and behavior: genetic aspects

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Summary. Three animal models, based on genetic differences in endogenous opioid peptides and opioid receptors, are described. Obese mice and rats, whose pituitary opioid content is elevated, may be used to investigate eating disorders. Recombinant inbred strains of mice, which differ in brain opioid receptors and analgesic responsiveness, can be used for study of opioid- and nonopioid-mediated mechanisms of pain inhibition. Individual reactivity to opioids can be examined in C57BL/6 and DBA/2 inbred strains of mice. A model that combines a variety of opioid effects is offered and suggests the existence of a genetically determined dissociation of opioid effects on locomotor activity and pain inhibition. In addition, stimulatory locomotor responses in the C57BL/6 reaction type are linked to a high risk of drug addiction and facilitatory effects on adaptive processes, while high analgesic potency in the DBA/2 reaction type is accompanied by a low proneness to drug abuse and amnesic properties of opioids.

Key words. C57BL/6, DBA/2 and CXBK mouse strains; genetically obese rodents; opioid receptors; opioid ligands; food intake; analgesia; locomotor activity; learning and memory; social stress; addiction.

One of the most exciting and stimulating new areas in the study of neuroregulation is that of the endogenous opioid systems. The discovery of the existence of opioid receptors in 1973 [83, 103, 106], and of the first endogenous opioid peptides, leu- and met-enkephalin, in 1975 [43], has elicited an extensive search for the physiological role of opioid systems. Pharmacological investigations using morphine and other opiate agonists on the one hand, as well as opiate antagonists such as naloxone, on the other, have implicated opioids in a wide variety of possible functions: pain modulation, respiration, appetite and temperature regulation, motor activity, sleep, learning and memory, and the states of reward and pleasure. Furthermore, opioids may regulate endocrine functions, and may be involved in the etiology of the major psychiatric disorders [1, 23, 28, 78, 104, 106]. In schizophrenic illness excessive or deficient opioid activity in the brain has been discussed [51, 58], and increased [7], or decreased [84] concentrations of β-endorphin-like peptides have been reported in the cerebrospinal fluid of schizophrenics. Alternatively, a derangement in the processing of endorphins, leading to an imbalance between β-endorphin fragments, has been suggested as an etiological factor in the psychopathology of schizophrenia [112]. Altered opioid mechanisms may also play a role in human epilepsy [24], as well as in manic-depressive illness [23, 51], and significantly higher cerebrospinal fluid opioid activity has been found during mania than during depression [84].

The present understanding of the role of endogenous opioids in psychopathology is fragmentary, and further attempts to elucidate the opioid function in normal and abnormal behavior are of fundamental importance. An advantageous approach to this goal is the use of genetically-defined animals which differ in their opioid peptides, opioid receptors, or in their responses to opiates. For this purpose genetically-determined animals can be obtained which are derived from breeding of mutants, recombinant-inbred lines and inbred strains, as well as by selective breeding from a heterogeneous population. The aim of this review is to summarize some of the recent findings on the genetics of the opioid systems and on possible links between opioids and behavior.

1. Genetics of the opioid systems

A large number of opioid peptides have been isolated and identified during the last ten years. In 1982, use of recombinant DNA technology revealed that all these opioids belong to three genetically distinct peptide families. One gene codes for pro-opiomelanocortin, the precursor of β-endorphin, as well as the nonopioid peptides ACTH, β-LPH and α-MSH. A second gene codes for pro-enkephalin, which contains four met-enkephalin sequences, two extended met-enkephalin peptides and a leu-enkephalin peptide. A third gene codes for the precursor pro-dynorphin which contains dynorphin A and B, as well as the neo-endorphin peptides (for details see Akil et al. [1]). As might be anticipated in the light of the above, the concept of multiple opioid receptors, already suggested in the mid-sixties [61, 85], was confirmed in bioassays, autoradiographic studies and opiate binding experiments using selective ligands and competitive displacement [93]. The existence of μ, δ, and κ-receptors was shown and a considerable amount of information accumulated, indicating that the different opioid peptides interact to varying degrees with these multiple receptors [52]. β-Endorphin has about equal affinities at the μ- and δ-binding sites, with negligible affinity at the κ-site. Met- and leu-enkephalin bind selectively at the δ-site and the dynorphins have high affinities at the κ-site. Genetic differences may exist at the ligand level (synthesis, release, modification and metabolism of the opioid peptides), at the receptor level (number and affinity of receptor sites), as well as in the processing of receptor stimulation.

1.1 Genetic differences at the opioid receptor level

The first report of a statistically significant strain difference in opiate binding was published in 1975 [2]. In those experiments stereospecific binding of naloxone was investigated in whole brain homogenates of seven recombinant-inbred lines of mice, which were derived by inbreeding from the F2 generation of a cross between C57BL/6By and BALB/cBy progenitor strains. Scatchard analysis revealed that CXBH had a significantly higher (+ 23%), and CXBK a significantly lower (− 28%) number of receptor sites when compared with all other recombinant-inbred lines (CXBD, CXBE, CXBG, CXBI, CXBJ), the progenitor strains C57BL/6By and BALB/cBy, and their reciprocal F1 hybrids (table 1). In subsequent papers [47, 92], lower binding to whole brain homogenates of CXBK mice was confirmed when the opiate antagonist naloxone, or the agonist dihydromorphine, were used, both of which preferentially bind to the μ-receptor type. Binding of the δ-agonist D-ala-D-leu-enkephalin, however, was found to be similar in all recombinant-inbred strains and in their progenitors C57BL/6By and BALB/cBy [92]. When binding of the putative κ-agonist ethylketocyclazocine was measured, contradictory results were obtained, in that the values for CXBK brain homogenates were about 20% lower [92], or higher [47], when compared to those of CXBH mice. Using semiquantitative autoradiographic analysis, incubation of brain slices with dihydromorphine or D-ala-D-leu-enkephalin revealed that the CXBK strain had either less, or the same amount, of μ-binding as the C57BL/6By strain in all brain areas examined [70], essentially confirming the above-mentioned studies with whole brain tissue. With regard to the δ-sites the two strains did not consistently differ, i.e., in some areas the CXBK mice had lower levels but

Table 1. Differences in opioid receptor binding of genetically-defined mice

Mice	Tissue	3H-Ligand	Low	Binding	High	Reference
Recombinant-inbred strains	Whole brain	μ-type:				
		Naloxone	CXBK < CXBJ, CXBI, CXBE, CXBD, CXBG, C57BL/6, BALB/c		< CXBH	2
		Naloxone, dihydromorphine	CXBK < CXBD, CXBE, CXBI, CXBH, C57BL/6 <		BALB/c	92
		Naloxone	CXBK	<	CXBH, C3H	47
		k-type:				
		Ethylketocyclazocine	CXBK < CXBH, CXBD, CXBE, CXBI, C57BL/6 <		BALB/c	92
			CXBH	< CXBK <	C3H	47
		δ-type:				
		D-ala-D-leu-enkephalin	Similar binding values for all recombinant-inbred strains, and for C57BL/6 and BALB/c			92
Inbred strains: C57BL/6, DBA/2	Striatum	μ-type:				
		Naloxone, dihydromorphine		DBA/2 = C57BL/b		90
			DBA/2	<	C57BL/6	73
		δ-type:				
		D-ala-met-enkephalin, Leu-enkephalin	DBA/2	<	C57BL/6	90
	Hippocampus	μ-type:				
		Naloxone	C57BL/6	<	DBA/2	73

in many areas they had levels equal to or greater than those for the C57BL/6By mice. With this detailed knowledge of differences in the opioid receptors available, CXBK mice may prove a useful tool to investigate which receptor type in a given brain structure mediates a specific effect of opioids (see section 2.2).

The experiments with recombinant-inbred strains have often included comparisons with well-defined inbred strains of mice, e.g., to the progenitors C57BL/6By and BALB/cBy[2,92], or to C3H[47]. No difference in naloxone binding to whole brain homogenates was found between C57BL/6By and BALB/cBy mice[2,92] and in experiments using the analogous strains from the Jackson Laboratory, specific binding at saturation level (B_{max}) did not differ among C57BL/6J, BALB/cJ, Swiss-Webster and ICR strain[22]. Interesting strain differences have been described between C57BL/6 and DBA/2 mice. A preliminary report assessed naloxone binding in membrane preparations of whole brain homogenates[31]. Naloxone binding sites of C57BL/6 mice had a lower B_{max}, but a higher affinity (K_d) when compared to DBA/2 mice. In a more detailed study, binding of 4 different ligands was measured in membrane preparations from striatum, brain stem, forebrain and neocortex of C57BL/6J and DBA/2J mice[90]. When δ-agonists were used, C57BL/6J mice showed higher striatal opioid receptor binding in comparison to DBA/2J mice (table 1). When μ-selective ligands were used, similar[90] or slightly higher[73] binding values were found in the striatum of C57BL/6 in comparison to DBA/2 mice. No differences were obtained between these strains with δ- or μ-ligands in other brain areas[73,90], except for a 20% lower naloxone binding in the hippocampus of C57BL/6 mice[73]. Scatchard analysis revealed, for the striatum, a 70% higher B_{max} of δ-binding sites in C57BL/6J as compared to DBA/2J mice, while the K_d was similar for these two strains[90]. This strain difference in binding of δ-selective ligands was eliminated after intraventricular injection of 6-hydroxydopamine, a neurotoxic agent for dopaminergic neurons, suggesting that the additional δ-receptors in the striatum of C57BL/6J mice were associated with dopaminergic neurons. A possible correlation between these binding data and specific effects of opiates in mice of the C57BL/6 and DBA/2 strains will be discussed in section 2.3.

In two lines of rats, RHA/Verh and RLA/Verh, selectively bred for rapid acquisition and non-acquisition of two-way avoidance, respectively, binding capacity of the preferential μ-ligand DAGO to striatal membrane preparations was significantly higher in RHA/Verh (B_{max} 9.60 $\pm$ 0.44 fmol/mg tissue; K_d 0.30 $\pm$ 0.04 nM), than in RLA/Verh rats (B_{max} 5.56 $\pm$ 0.35 fmol/mg tissue; K_d 0.36 $\pm$ 0.08 nM). In contrast, no differences were obtained with the preferential δ-ligand D-ala-D-leu-enkephalin in the striatum, as well as with μ- and δ-ligands in hippocampal membrane preparations[55].

1.2 Genetic differences at the opioid ligand level

Differences in levels of endogenous opioid ligands have also been reported for different inbred strains of mice (table 2). The content of met-enkephalin in the striatum of C57BL/6J was significantly lower (about 40%) than in that of DBA/2J mice[3]. C57BL/6J mice were found, likewise, to possess lower levels of whole brain met-enkephalin as compared to DBA/2J and C57BL/6N[5]. However, for 6 brain regions studied, no significant differences were found between these strains of mice. In another series of experiments, inbred strains of mice differed significantly in resting pituitary content of β-endorphin-like immunoreactivity[20]. The maximum difference among 16 strains was about 3-fold, e.g., pituitary β-endorphin-IR in C57BL/6N and C3H/HeN was higher than in DBA/2N or BALB/c mice. However, opposite results were found when pituitary contents of C57BL/6J and DBA/2J were compared[54]. Further experiments[32,33] pinpointed the elevated pituitary β-endorphin-IR of C57BL/6N as a 2–2.5-fold higher content in the neurointermediate lobe, when compared to BALB/c and DBA/2N mice. Content of β-endorphin-IR in the anterior lobe of the pituitary was similar in C57BL/6N and DBA/2N mice[32], or even higher in the DBA/2N strain[33]. The serum β-endorphin-IR reflected the strain differences in the neurointermediate lobe. No differences were found in the hypothalamic content[32,54], while higher levels of β-endorphin-IR were measured in the amygdala and periaqueductal grey of the C57BL/6J strain when compared to DBA/2J[54].

Differences in pituitary β-endorphin-IR were also found in genetically obese rats and mice, as compared to their lean littermates (table 2). The pituitaries of the Zucker 'fatty' rat (*fa/fa*) and of the obese mouse (*ob/ob*) contained about twice as much β-endorphin as did controls[60]. A greater than threefold increase of β-endorphin-IR was found also in plasma of *fa/fa* rats. Subsequent studies[36,96] confirmed the elevated β-endorphin-IR in pituitaries from obese mice. In addition, increased levels of leu-enkephalin and dynorphin were found in the neural part of pituitaries from obese mice[26,86]. However, the pituitary was the only structure that showed differences in the content of endogenous opioid peptides. β-En-

Table 2. Differences in levels of opioid ligands of genetically-defined animals

Animals	Tissue	Endogenous Ligand	Low	Content	High	Reference
Inbred strains	Striatum	Met-enkephalin	C57BL/6	<	DBA/2	3
of mice:	Whole brain	Met-enkephalin	C57BL/6	<	DBA/2	5
C57BL/6, DBA/2	Pituitary	β-endorphin	DBA/2	<	C57BL/6	20
			C57BL/6	<	DBA/2	54
	Pit: neuroint. lobe	β-endorphin	DBA/2	<	C57BL/6	32, 33
	Pit: anterior lobe	β-endorphin		DBA/2 ~ C57BL/6		32
			C57BL/6	<	DBA/2	33
	Amygdala, periaqueductal grey		DBA/2	<	C57BL/6	54
Obese mutants:						
Obese mice (*ob/ob*)	Pituitary	β-endorphin	Lean	<	*ob/ob*	36, 60, 96
		Leu-enkephalin	Lean	<	*ob/ob*	96
		Dynorphin	Lean	<	*ob/ob*	26
Fatty rats (*fa/fa*)	Pituitary	β-endorphin	Lean	<	*fa/fa*	60
Diabetic mice (*db/db*)	Pituitary	β-endorphin	Normal	<	*db/db*	37
		Met-enkephalin	Normal	<	*db/db*	37

dorphin, leu- and met-enkephalin, as well as dynorphin in the hypothalamus and other brain structures, were all unchanged in obese mice when compared to lean littermates [36, 60, 67, 96, 97]. Similarly, a recent paper [37] reported for pituitaries of obese diabetic mice (*db/db*) a 5.4-fold increase of β-endorphin-IR and a 1.6-fold elevation of met-enkephalin-IR. Again, no differences were found in the hypothalamic contents of these opioid peptides, when compared with those in control animals.

The following section will focus on the description of three animal models for the investigation of genetically determined differences in opioid-mediated behaviors.

2. *Genetic differences in opioid-mediated behaviors*

2.1 *Eating disorders – the genetically obese rat and mouse*

There is a large body of evidence suggesting a role for the endogenous opioids in the central and peripheral regulation of food-intake; injections of opiate agonists and opioid peptides generally induce feeding, while opiate antagonists have anorexic properties. Furthermore, an activation of the endogenous opioid peptides may be involved in stress-induced eating, a well-known phenomenon in animals and humans [68, 91] and endorphin pathways may play a significant role in obesity, as well as other eating disorders, such as anorexia nervosa or bulimia [34]. Classical animal models of obesity, such as the obese mouse (*ob/ob*) and the Zucker 'fatty' rat (*fa/fa*), both syndromes caused by recessive mutations of a single gene, show higher baselines of food intake when compared to their lean littermates, and naloxone has been shown to suppress eating more effectively in obese, than in control, animals [60]. The same findings have been obtained in experiments with obese diabetic mice (*db/db*) [56]. On the other hand, obese mice were more resistant to the feeding-inducing properties of kappa opiate agonists, such as butorphanol or tifluadom [67], suggesting a possible decrease in κ-receptor sensitivity. In considering these differences it should be remembered that while obese animals receive a higher absolute amount of drugs because of their greater body weight [60], their absorption of drugs after subcutaneous administration may be less effective because of their excessive stores of adipose tissue [67]. Thus, differences in blood levels, and in the effective dose at relevant receptors, may be important. A causal relationship between elevated levels of pituitary opioid peptides and the obesity syndrome is at least questionable, since it appears that increased β-endorphin concentrations may be an epiphenomenon secondary to the development of obesity [96]. Quaternary naltrexone methobromide and N,N-diallyl-normorphine bromide which, in contrast to naltrexone and N-allyl-normorphine, do not cross the blood-brain barrier, have failed to suppress food intake [59] and water intake [79] in obese rodents, suggesting that opioids may regulate consummatory behavior via central receptors. Further work is required to examine mutant obese animals for abnormalities in their brain opioid systems. Since normal levels of opioid peptides were found so far in the hypothalamus [36, 60, 67, 96] and other brain regions [36, 67] of these mutants, future experiments should study the brain opioid receptors in these obese animals. A more profound understanding of these classical animal models of obesity might be helpful for treatment and control of certain forms of overeating and food addiction in humans. For example, the possibility that obesity may result from autoaddiction to endogenous opioid peptides [66], and that the drive to eat between meals may be a correlate of a withdrawal symptom, has been discussed. In contrast, for anorexia nervosa, the question has been asked whether anorectics may lack the endogenous reward system [34], while the increased levels of opioids found in their cerebrospinal fluid during periods of minimum body weight [84] have been attributed to the stress of drastically-reduced caloric intake.

2.2 *Mechanisms of pain – analgesia in CXBK mice, a μ-receptor-deficient strain*

Research over the past decade has revealed evidence for the role of multiple neural systems in modulation of pain transmission. Much of this work has focused on endogenous opioid systems, and on their interactions with nonopioid mechanisms [1, 105]. Clinically, the main problems in controlling pain are the addictive properties of most analgesics, and marked individual differences in the efficacy of opiate drugs and pain therapies that act via endogenous opioid release [69]. Interestingly, differential activation of opioid and nonopioid analgesic systems has been reported in an animal model, using diverse paradigms of electric footshock as a stressful manipulation [113]. A promising approach to studying the possibility of pain inhibition by nonopioid mechanisms is the use of the CXBK mouse strain. These mice are particularly deficient in high affinity μ-sites in many areas involved in pain processing, including the ventral periaqueductal grey matter, raphe nuclei, and spinal cord [70].

When the nociceptive threshold of CXBK mice was measured, their baseline response latencies in the tail-flick and hot-plate tests were lower than those of the other recombinant-inbred lines, or obese mutants [97, 100]. In addition, morphine (2.5, 5 and 10 mg/kg) elicited a significantly smaller analgesic effect in CXBK mice when compared to other recombinant-inbred lines of mice and to their progenitor

strains BALB/c and C57BL/6By[2,48,97]. The correlation between the amount of receptors in whole brain and the analgesic response was positive but not statistically significant, suggesting that differences in the B_{max} of opioid receptors in brains of recombinant-inbred strains of mice were not enough to account for the genetic differences in their analgesic responsiveness[2]. In a study restricted to three strains, however, the antinociceptive activity of morphine measured by the hot-plate and tail-flick assays, ranked in CXBK, CXBH and C3H mice in the same order as their binding capacities for 3H-naloxone in whole brain[47]. While there are conflicting data[4], a quite different approach again demonstrated a relationship between differences in nociception and differences in opioid receptor binding; rearing in social isolation decreased the B_{max} of opioid receptors in C57BL/6 and increased it in Swiss Albino mice, and the housing-induced change in the analgesic response to morphine correlated, in these animals, with their changes in B_{max}[6].

Among a variety of noxious, aversive stimuli that are capable of rendering an organism analgesic, mice subjected to social conflict and defeat exhibit a marked antinociception. Its opioid mediation has been shown by cross-tolerance to, and from, morphine[65,95], as well as by the antagonistic potency of naloxone, naltrexone and β-chlornaltrexamine[64,95,101,107], and its central origin has been demonstrated[64,95]. Concerning strain differences in the phenomenon of social conflict-induced analgesia, it was shown that exposure to 70 bites increased tail-flick latencies in the μ-receptor deficient CXBK mice by only 1 second, while the increase in C57BL/6J and BALB/cBy was 2 s, in C57BL/6By 4 s, and, in the DBA/2J and B6AF1 strain, 6 s[64]. The low and high degree of social conflict-induced analgesia in CXBK and B6AF1 mice, respectively, appears to correlate very well with the strain-specific sensitivity to the analgesic effect of a given dose of morphine[65]. Similarly, poor electropuncture analgesia was also reported for the CXBK strain[82]. When inescapable footshock was used as an aversive stimulus, CXBK mice showed a short-lasting, naloxone-insensitive analgesia, while in the C57BL/6By strain opioid and nonopioid pain inhibitory systems were activated, depending upon the parameters of footshock administered[69]. Analogous strain differences in the activation of opioid and nonopioid analgesia systems following footshock exposure were also reported for eight strains of rats[108,109]. Similarly, naloxone reduced post-swim analgesia in mice selectively bred for high stress-induced increase in hot-plate response latency, while the antagonist was ineffective in the low-responding line[80,81].

Thus the CXBK mice, which are relatively deficient in the μ-receptor, in comparison with other recombinant-inbred lines, or inbred strains of mice, are very useful for the investigation of pain inhibitory mechanisms. The low analgesic effects of morphine and of diverse stressful manipulations might be of special interest in CXBK mice. But for a better understanding of these peculiarities, the content of opioid peptides in relevant brain regions should also be measured before, and after, exposure to footshock or defeat-stress. From the clinical point of view, and especially with regard to those patients who respond poorly to opioid-mediated pain therapies, the CXBK strain may represent a useful model for testing new methods of pain inhibition via nonopioid pathways.

2.3 Differential reaction types to opioids – the C57BL/6 and DBA/2 inbred strains of mice

It is well known that human individuals may differ in their responses to drugs[57], as well as in their responsiveness to stress, and in coping with strategies used to deal with stressful events[50]. With regard to opioids, substantial individual variability exists, e.g., in excitant and depressant effects of morphine, and in the liability to drug addiction (for review, see Martin[62]). Some individuals stop self-administration of narcotic analgesics after experience, while others become compulsive drug users. For those at high risk of addiction, drugs may have less negative side- or after-effects, or increased euphoric and reinforcing action. It has been claimed that euphoric effects of morphine predominate in 'nondependent' postaddicts, while sedative effects predominate in nonaddicts. To understand the neurophysiological and neurochemical bases of individual differences in the effects produced by opioids, and by stress known to coactivate the opioid systems, inbred strains of mice may prove a useful model.

2.3.1 The dissociation between running and analgesia

A negative correlation between the degree of running and analgesic response to morphine was evident when the inbred strains C57BL/6J, DBA/2J and BALB/cJ, as well as their reciprocal F1 progeny and backcrosses, were compared[13]. After intraperitoneal injection of morphine, mice of the C57BL/6J strain showed the highest increase in locomotor activity, while they were the least sensitive to the analgesic effect (table 3). In contrast, DBA/2J mice were more sensitive to the analgesic effect of morphine, but the drug did not enhance their locomotor activity. Similar results were obtained after intracerebroventricular injection of morphine[29]. Intra-hippocampal injection of the opiate antagonist naloxone[111], as well as of the antiserum to met-enkephalin[110], caused an increase of various exploratory activities in DBA/2J and depressed the scores in C57BL/6J mice. The inheritance of morphine-induced running response and analgesia in the F1 progeny of reciprocal C57BL/6J × DBA/2J crosses was found to be regulated by incomplete dominance of the C57BL/6J morphine effects[13]. In contrast, DBA/2N were partially dominant over C57BL/6N mice in terms of two other morphine-induced responses, i.e., increase in plasma cyclic nucleotides and hypothermia[72,74]. The negative correlation between the effects of opioids on locomotor activation and the effects on analgesia was confirmed in a study with lines of mice selectively bred for high running, or non-running, response to a 20 mg/kg dose of levorphanol[49]. The line selected for low locomotor response to levorphanol showed a more pronounced analgesic drug effect than the heterogeneous stock from which selective breeding was started.

Several studies have reported different responses of C57BL/6 and DBA/2 mice to a variety of opioid ligands, and have led to hypotheses concerning the mechanisms responsible for preferential opioid-induced locomotor activity and analgesia in these strains. Buprenorphine, an opioid with mixed, agonist-antagonist actions and high relative affinity to the μ-binding site, induced analgesia in DBA/2N mice and the running response in C57BL/6N mice, but antagonized only the morphine effect on locomotor activity in the C57BL/6N strain[27]. Beta-funaltrexamine, a long-acting opiate antagonist that preferentially binds to the μ-receptors, blocked morphine-induced analgesia in DBA/2J mice and locomotor activation in C57BL/6J mice, and stimulated the locomotor activity of C57BL/6J mice[30]. The analgesic response to methionine-enkephalin, which mainly acts via δ-receptors, was less pronounced in C57 than in DBA mice[29]. κ-Agonists, like tifluadom, ethylketocyclacozine and bremazocine depressed locomotor activity in C57BL/6 and DBA/2 mice[14,17,38], although the C57 strain showed a lower sensitivity for κ-ligands in some experiments[88,89]. Interestingly, κ-agonists, which decreased locomotor activity in C57BL/6 and DBA/2 mice, were shown to inhibit the release of dopamine from slices of rat striatum[71]. Morphine, on the other hand, increased striatal dopamine release and stimulated locomotor activity in C57BL/6J mice, while it inhibited striatal

Table 3. Differential effects of opioids (injected, or released by stress) in mice of the inbred strains C57BL/6 and DBA/2

Behavior	Treatment	C57BL/6	DBA/2	Reference
Locomotor activity	Morphine	Increased locomotion	No effect, or decrease	13, 29
	κ-agonist: Tifluadom, ethylketocyclazocine, bremazocine	Decreased locomotion	Decreased locomotion	14, 17, 38
Analgesia	Morphine, met-enkephalin	Low analgesia	High analgesia	13, 29
	Social conflict	Low analgesia	High analgesia	53, 54, 64, 102
Learning and memory	Heroin, dermorphin, D-ala-D-leu-enkephalin (posttrial, high dose)	Improved performance	Impaired performance	10, 15
	Naloxone (posttrial)	Impaired performance	Improved performance	11
	Post-trial immobilization	Improved performance	Impaired performance	18
	Increasing experience of social conflict	Increased behavioral adaptation Change in behavioral strategy	Decreased behavioral adaptation Unchanged behavioral strategy	102 53
Proneness to drug addition	Morphine drinking	Preference for sweetened morphine solution	Avoidance of sweetened morphine solution	42
Withdrawal in dependent mice	Naloxone-induced withdrawal	High sensitivity	Low sensitivity	8, 39

dopamine release in DBA/2J mice and either did not affect, or depressed, their locomotor activity [87]. The importance of catecholamines for morphine-induced locomotor activation in C57BL/6J mice was also demonstrated in animals that had been pretreated with the catecholamine depletor α-methyl-p-tyrosine [12]. Morphine did not elicit locomotor activation in these pretreated C57BL/6J mice, and their analgesic response was not affected. Septal lesions, however, known to reduce acetylcholine levels in the brain areas that receive cholinergic input from the septum, antagonized morphine-induced analgesia but not the running response [12]. Therefore, cholinergic mechanisms seem to play an important role in analgesia, while catecholaminergic systems are involved in the locomotor effects of opiates (see reviews by Oliverio et al. [77, 78]). Interestingly, additional δ-receptors were found in the striatum of C57BL/6J when compared to DBA/2J mice (see section 1.1), and it was suggested that they might be important for the opiate-induced locomotor stimulation [90]. The concept of associating a certain morphine effect with a specific receptor subtype, however, is an oversimplification. This is supported by the findings that intracerebroventricular injection of met-enkephalin, a preferential δ-agonist, did not induce a running response in C57BL/6J mice [29], and that β-funaltrexamine, a preferential μ-antagonist, blocked morphine-induced analgesia as well as the locomotor stimulation [30]. It must also be mentioned, in this context, that morphine decreased locomotor activity in RHA/Verh and RLA/Verh rats and induced an equal antinociceptive response in both lines, although they differed in their striatal μ-receptors [55].

2.3.2. Learning and memory

β-Endorphin is released during exposure to a variety of novel situations including aversive conditioning paradigms, as demonstrated by the decreased β-endorphin immunoreactivity in the brain following these exposures [45]. Therefore, it is not surprising that during the last 10 years studies from different laboratories have shown that opiate agonists and antagonists, as well as endogenous opioid peptides, affect centrally mediated processes such as learning and memory [63].

The genetic approach to investigating the role of opioids in learning and memory has focused mainly on the use of inbred strains of mice. In addition to the differences previously enumerated in brain opioid receptor population, pituitary β-endorphin content, brain enkephalin levels and responses to morphine, C57BL/6 and DBA/2 mice also differ in the effects of opiates on learning and memory (table 3). Immediate post-trial administration of high doses of heroin (5 mg/kg, i.p.) improved performance of C57BL/6N mice in a pattern discrimination test, and impaired that of DBA/2N mice [10]. Low doses (0.5 mg/kg, i.p.), however, given either before or after each training session, improved performance in both strains. Subsequently [11], using the same test, the opiate antagonist naloxone (5 mg/kg, i.p.) impaired performance in C57BL/6N mice and improved that of DBA/2N mice, when injected immediately after each training session. These strain-dependent effects of naloxone were confirmed in the Y-water maze, when the mice were trained to swim toward the dark alley. Pretrial administration of morphine (1.5 mg/kg, i.p.) facilitated two-way active avoidance responding in mice of the C57BL/6J strain subjected to three daily sessions of 50 trials in the shuttlebox, while the drug did not influence the footshock avoidance of DBA/2J mice [21]. Recently [15], dermorphin and D-ala-D-leu-enkephalin, relatively selective opioid ligands for the μ- and δ-receptors, respectively, were administered intracerebroventricularly (i.c.v.) to C57BL/6N and DBA/2N mice immediately after training in a step-through passive avoidance test. Both peptides, irrespective of their different receptor selectivity, had the same effect, i.e., a low dose of 5 ng impaired retention performance of both strains, whereas a high dose of 50 ng blocked retention in DBA/2N mice completely, but improved it in C57BL/6N mice. All of these dose- and strain-dependent effects were antagonized by a dose of naloxone (0.3 μg, i.c.v.) which was ineffective when given alone. It appears, therefore, that opioid agonists at low doses have similar effects on learning and memory in C57BL/6 and DBA/2 mice, while at higher doses strain-dependent opposite effects are obtained, i.e., facilitation in C57BL/6 and impairment in DBA/2 mice.

Recent experiments in C57BL/6 and DBA/2 mice have suggested that the stress-induced release of endogenous opioid peptides causes genotype-dependent effects on learning and memory analogous to those found after administration of opiate agonists (table 3). Immobilization in a restraining tube for a period of 60 min, immediately after training in a step-through task, improved the retention-performance of C57BL/6N mice and impaired that of DBA/2N mice in the passive avoidance situation [18]. Both the positive and the negative effects of stress were antagonized by a dose of naloxone (1 mg/kg, i.p.) which was ineffective on its own, suggesting that the effects were mediated through opioid receptors. Additional experiments demonstrated that immobilization stress in DBA/2N mice induced an opioid-mediated analgesia [86], and also enhanced the effects of morphine on nociception and learning [16, 86].

In a series of experiments in C57BL/6 and DBA/2 mice, social conflict was used as an aversive stimulus which elicited, on the one hand, endogenous analgesia and, on the other hand, behavioral responses such as escape and submissive postures. Clear strain differences in the extent of social conflict-induced analgesia were obtained [53, 54, 64, 102]. With increasing number of bites received (either 10, 30, 50 or 70 bites), the response latencies measured with the hot-plate, or tail-flick tests increased significantly in DBA/2J mice while no [53, 102] or only low [54, 64] encounter-induced analgesia was found in C57BL/6J mice. In one study [53], mice with social conflict experience of 50 bites were exposed to a second and third aggressive confrontation on subsequent days and their change in behavior, compared to the first encounter, was taken as an index of learning. In mice of the C57BL/6J strain, escape behavior significantly decreased during repeated aggressive confrontations of 50 bites, while defensive upright postures increased. In contrast, DBA/2J mice did not modify their behavioral response pattern, and they showed a significant stress-induced analgesia after the first, second and third encounters. In another study [102], mice of both strains were exposed 24 h after experience of a single aggressive confrontation with a nonaggressive opponent, and learning was measured in terms of escape behavior and submissive postures displayed upon mere contact. These responses were not shown in animals without aggressive encounter experience (0 bites). Experience of social conflict with different numbers of bites received (10, 30 or 50 bites) increased the recall of learned responses in C57BL/6J mice in a rate-dependent manner. DBA/2J mice, on the other hand, showed a marked social conflict-induced analgesia, and retention of learned behavior decreased as a function of aggressive encounter intensity. It is suggested that the social conflict-induced analgesic state in DBA/2J mice might have modified retention of submissive behavior. Thus, mice of the C57BL/6 and DBA/2 strains, exposed to stressful situations, react differently. As the aversive stimulus is increased (e.g. to 50 bites, or repeated aggressive confrontations), C57BL/6 mice increase their conditioned behavioral responses and modify their defense strategy, while in DBA/2 mice the stress-induced antinociceptive response protects the animal from the aversive stimulus while, at the same time, interfering with behavioral adaptation. The idea that stress-induced analgesia may interfere with behavioral adaptation in an aversively-motivated learning task is supported by the recent findings showing that Long Evans rats, selectively bred for low avoidance performance in a shuttlebox, exhibited a more profound analgesic response following inescapable electric shock, when compared to animals selectively bred for high avoidance performance [75].

2.3.3 Opiate addiction

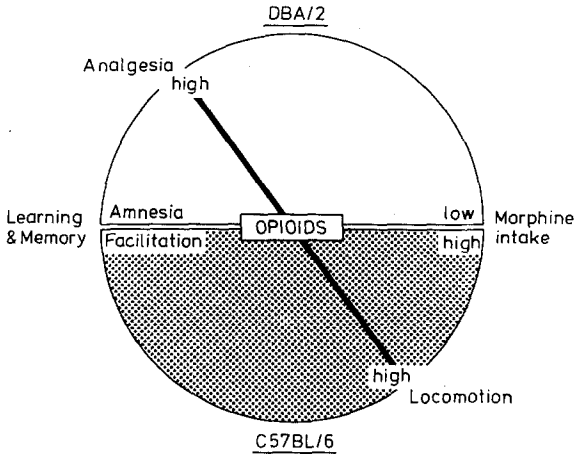
The importance of genetic factors in proneness to drug addiction was shown in a classical paper [76], where two lines of Sprague-Dawley rats were selectively bred for quantitative differences in morphine drinking. Over three generations, the addiction-susceptible S-line increased morphine drinking after a 14-day period of daily morphine injections, while the addiction-resistent R-line showed slightly decreasing scores. Likewise, addiction-prone and addiction-resistant lines have been found after periods of forced morphine intake among different strains of rats selectively bred for other criteria [40, 98], as well as among inbred strains of mice (table 3). C57 showed a higher morphine consumption when compared to CBA mice [25], and C57BL/6J preferred a morphine solution (0.375 mg/ml) containing saccharin, while DBA/2J mice avoided the same drug solution [42]. 11 of 40 C57BL/6J mice even drank lethal amounts of the sweetened morphine solution. Interestingly, addiction-prone and -resistant lines of rats and mice, characterized by different rates of morphine drinking, showed a similarly differential tendency toward alcohol intake [5, 25, 42, 76, 99, 114]. While there are conflicting data [40, 114], the bulk of evidence suggests a close parallel between proneness to opioid dependence and ethanol consumption.

The link between genetically-determined differences in the opioid systems and morphine addiction liability is unclear. However, the level of whole brain met-enkephalin among various mouse strains has been found to be negatively correlated with their voluntary alcohol consumption [5] which, as mentioned above, often paralleled morphine intake. Thus, mice of the C57BL/6J and C58/6J strains, which preferred ethanol solution over water, had significantly lower brain met-enkephalin levels than DBA/2J mice, which preferred water over ethanol. Gianoulakis and Gupta [32, 33], on the other hand, suggested that high levels of β-endorphin in the pituitary and plasma, as well as the ability to increase the release of β-endorphin from the pituitary and hypothalamus, might be associated with the genetically determined high ethanol consumption in C57BL/6N mice, as compared to BALB/c and DBA/2N mice. C57BL/6 mice may, therefore, provide a useful model for the human situation, where met-enkephalin has been found to be decreased in cerebrospinal fluid and plasma of heroin addicts, while plasma levels of β-endorphin were elevated [19].

Other evaluations of opiate dependence have quantified the severity of withdrawal symptoms in lines of rats and mice selectively bred for other criteria [35, 41], and among different inbred strains of mice (table 3). The incidence of abrupt withdrawal jumping, observed 6 h after removal of morphine pellets which had been implanted for three days, was high in C57BL/6N, intermediate in C3H, Swiss, ICR and A/J strains, and very low in DBA/2J mice [8]. Similar strain differences were reported for naloxone-precipitated withdrawal jumping 24 h after implantation of a morphine pellet, i.e., the ED_{50} values of naloxone were 0.33 mg/kg for C57BL/6N and 4.38 mg/kg for DBA/2J mice, while the corresponding values for Swiss, C3H, ICR and A/J strains laid between these extremes. These findings were confirmed after prolonged exposure to morphine or ethylketocyclazocine, when the ED_{50} values for naloxone-precipitated withdrawal jumping were found to be significantly lower in C57BL/6J (0.24 and 0.27 mg/kg) when compared to DBA/2J mice (0.43 and 0.68 mg/kg), respectively [39]. Similarly, low doses of naloxone (0.2–1.0 mg/kg) induced withdrawal jumping in ICR, Swiss and C57BL/6J mice, while BALB/cJ mice showed almost no naloxone-precipitated jumping even after a high dose (10 mg/kg) of the antagonist [22].

2.3.4 The C57BL/6 and DBA/2 reaction types – facts and speculations

Based on the dissociation between opioid-induced analgesia and locomotor activation, the opposite effects of opioids on learning and memory, and differential proneness to opiate addiction in C57BL/6 and DBA/2 mice, a model is offered to characterize the differential reactivity of these strains to opioids (fig.). In DBA/2 mice, characterized as 'analgesic type' (upper semicircle), opioids have high antinociceptive potency, they impair learning and memory processes in aversively-motivated situations demanding behavioral adaptations, and their addictive properties are low in terms of voluntary morphine intake and withdrawal severity. In C57BL/6 mice, characterized as 'locomotor type' (lower semicircle), opioids induce a locomotor activation, they have low analgesic potency, and facilitate learning and memory processes. This mouse strain is highly susceptible to drug addiction and proneness to drug dependence seems to be linked to the low level of brain met-enkephalin, and/or high levels of pituitary



Interaction between various effects of opioids in the 'analgesic DBA/2 type' (upper semicircle), and in the 'locomotor C57BL/6 type' (lower semicircle).

and plasma β-endorphin[5,32]. Interestingly, the marked withdrawal severity in terms of jumping incidence was found to be linked to high morphine-induced locomotor stimulation[8], indicating the possibility that both behaviors may be mediated by the same neuronal pathways.

When exposed to social conflict, DBA/2 mice reacted with a pronounced, intrinsic and opioid-mediated analgesia which, in turn, may have interfered with the processing of the aversive experience, measured in subsequent aggressive and nonaggressive confrontations[53,102]. Furthermore, the occurrence of a long-term analgesic reaction has been reported in attacked DBA/2J mice[101], which may represent a correlate of inescapable aversive experience and learned helplessness[46]. Thus, stimulation of endogenous opioid systems in DBA/2 mice predominantly activated protection mechanisms, which dampened the physical and psychic distress induced by bites. In contrast, C57BL/6 mice, showing only a small antinociceptive response upon increasing bite stimuli, modified their defense strategy and increased behavioral adaptation[53,102]. This active, behavioral coping with the aversive situation may have been due to the stimulation of different opioid pathways. Interestingly, strain-dependent stimulation and inhibition of dopaminergic systems by opioids[87], and by immobilization stress[9], known to be mediated by endogenous opioids, have been reported in C57BL/6 and DBA/2 mice, respectively. The limbic dopamine system, on the other hand, is known to be involved in opiate reward processes[44]. One may thus speculate that the two strains may therefore represent two extremes with regard to the functional aspects of released opioids, with a prevalence of opioids on reward mechanisms in the C57BL/6 locomotor type and a prevalence of opioids on distress-reducing protection mechanisms in the DBA/2 analgesic type. In view of the development of psychic dysfunctions, it would be interesting to observe these two strains of mice in chronic, uncontrollable stressful situations. It might be expected that the analgesic DBA/2 type would initially engage in a stable behavioral pattern, followed by a change of strategy at the moment its internal protective system breaks down. The locomotor C57BL/6 type would initially regulate via behavioral adaptation, and therefore, in a later phase, may be more susceptible to masking reactions and behavioral surrogate actions, which may be manifested by overreating, alcohol drinking, or narcotic drug abuse. Thus, the C57BL/6 and DBA/2 strains may be helpful in modelling human individual differences in relation to opioid-mediated responses, such as pain inhibition, reward mechanisms, memory processes, locomotor activation, drug-seeking behavior and withdrawal severity, as well as their mutual interaction. Furthermore, they may be of value with regard to investigating individual risk for psychopathological disorders induced by chronic, uncontrollable situations.

Acknowledgment. The authors wish to thank Dr David Blizard for many helpful suggestions.

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