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The Role of Functional Postsynaptic NMDA Receptors in the Central Nucleus of the Amygdala in Opioid Dependence

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Abstract

Activation of ionotropic *N*-methyl-D-aspartate (NMDA)-type glutamate receptors in limbic system nuclei, such as the central nucleus of the amygdala (CeA), plays an essential role in autonomic, behavioral, and affective processes that are profoundly impacted by exposure to opioids. However, the heterogeneous ultrastructural distribution of the NMDA receptor, its complex pharmacology, and the paucity of genetic models have hampered the development of linkages between functional amygdala NMDA receptors and opioid dependence. To overcome these shortcomings, high-resolution imaging and molecular pharmacology were used to (1) Identify the ultrastructural localization of the essential NMDA-NR1 receptor (NR1) subunit and its relationship to the mu-opioid receptor (μ OR), the major cellular target of abused opioids like morphine, in the CeA and (2) Determine the effect of CeA NR1 deletion on the physical, and particularly, psychological aspects of opioid dependence. Combined immunogold and immunoperoxidase electron microscopic analysis showed that NR1 was prominently expressed in postsynaptic (i.e., somata, dendrites) locations of CeA neurons, where they were also frequently colocalized with the μ OR. A spatial-temporal deletion of NR1 in postsynaptic sites of CeA neurons was produced by local microinjection of a neurotropic recombinant adeno-associated virus (rAAV), expressing the green fluorescent protein (GFP) reporter and Cre recombinase (rAAV-GFP-Cre), in adult “floxed” NR1 (fNR1) mice. Mice with deletion of NR1 in the CeA showed no obvious impairments in sensory, motor, or nociceptive function. In addition, when administered chronic morphine, these mice also displayed an acute physical withdrawal syndrome precipitated by naloxone. However, opioid-dependent CeA NR1 knockout mice failed to exhibit a conditioned place aversion induced by naloxone-precipitated withdrawal. These results indicate that postsynaptic NMDA receptor activity in central amygdala neurons is required for the expression of a learned affective behavior associated with opioid withdrawal. The neurogenetic dissociation of physical and psychological properties of opioid dependence demonstrates the value of combined ultrastructural analysis and molecular pharmacology in clarifying the neurobiological mechanisms subserving opioid-mediated plasticity.

I. Introduction

Opioid use can lead to an interrelated complex of adverse neural and behavioral adaptations that include tolerance, addiction, and dependence (Christie, 2008). Dependence, the focus of this paper, is typically studied within the framework of the withdrawal state produced by cessation of opioid exposure. However, dependence can also have an impact that endures beyond the immediate experience of withdrawal. One persistent effect of dependence is the result of learned associations between the noxious affective properties of withdrawal and

environmental cues (O'Brien, 2008), a topic that is an important theme of this chapter. Although the neural mechanisms subserving the complex aspects of dependence are far from clear, there is reason to believe that interactions between the ionotropic *N*-methyl-D-aspartate (NMDA)-type glutamate receptor and the mu-opioid receptor (μ OR), the major target of abused opioids like morphine, play a key role in opioid-adaptive processes. Moreover, the central nucleus of the amygdala (CeA), a critical coordinator of behavioral and emotional processes impacted by opioid exposure (LeDoux, 2000; Phelps and LeDoux, 2005), is a key anatomical substrate of NMDA-dependent forms of neural plasticity that may parallel the development of drug dependence (Hyman *et al.*, 2006). After providing some important background information, this paper describes recent results characterizing the synaptic organization and functional interactions of NMDA and μ ORs in the CeA, particularly with respect to opioid withdrawal. The implications of these results are considered within the context of prior neuropharmacological data on the role of the CeA in psychological and physical aspects of opioid dependence.

II. Opioids and Dependence

Opioids have been used by humans for millennia (Brownstein, 1993; Scarborough, 1995), and their analgesic properties have made them an important part of current medical care (Inturrisi, 2002). Despite their utility, the complex relationships between opioid pharmacology, use history, genetic factors, and other physiologic and environmental variables present significant problems with respect to their rational clinical use (Kreek, 2008; O'Brien, 2008; Weaver and Schnoll, 2002).

A pernicious complication of opioid consumption, dependence reflects the development of opioid-mediated adaptations that are expressed after termination of drug action by drug withdrawal-induced counter-adaptations, which are typically contrary to the acute effects of the opioid (Christie, 2008; Koob, 2009; Williams *et al.*, 2001). Although it has been reported that only a single opioid exposure may be required (Eisenberg and Sparber, 1979), repetitive use results in a more pronounced degree of dependence (Way *et al.*, 1969). The development of dependence on the prototypic opioid morphine requires functional μ ORs (Matthes *et al.*, 1996), and its severity is significantly related to genetic factors (Kest *et al.*, 2002; Korostynski *et al.*, 2007).

Well characterized in humans, dependence has been studied extensively in rodent models (Martin *et al.*, 1963). Opioid antagonist-precipitated withdrawal is the most common means of studying this phenomenon in animals, and will be the method discussed throughout the remainder of this chapter. Symptoms of opioid withdrawal can be elicited in dependent animals upon peripheral administration of general opioid receptor antagonists, such as naloxone or naltrexone (Hamlin *et al.*, 2001). However, intraventricular (Maldonado *et al.*, 1992) or intracranial (Stinus *et al.*, 1990) microinjection of opioid blockers can reproduce most withdrawal signs, indicating that dependence is critically mediated by diverse neural circuits. A list of some prominent withdrawal symptoms is provided in Table 8.1. As would be expected from the highly unpleasant nature of these symptoms, avoiding or relieving withdrawal is an important factor in continued drug use (Koob, 2009).

In addition to its acute unconditional effects, opioid withdrawal has other consequences that may endure beyond periods of detoxification and abstinence due to learned associations between the withdrawal state and environmental cues (O'Brien, 2008). Indeed, animals will learn to spend less time in a previously neutral environment paired with aversive periods of naloxone-precipitated withdrawal (Stinus *et al.*, 1990), a phenomenon termed conditioned place aversion (CPA). CPA is a species of classical conditioning whereby a conditional stimulus, such as a particular location with salient cues (i.e., tactile, visual, or other sensory stimuli), acquires secondary negative qualities, due to its pairing with an unconditional stimulus, such as opioid withdrawal (Cunningham *et al.*, 2006). Because the conditioned stimulus can evoke a response similar to the unconditioned stimulus, animals engage in avoidance behavior when subsequently exposed to the aversive conditioning environment.

What is notable about CPA is that it can be produced with doses of naloxone that are so low even physical withdrawal signs are not elicited (Gracy *et al.*, 2001). The latter finding indicates that a negative affective state (Schulteis *et al.*, 1998), rather than physical symptoms *per se*, is the requisite unconditioned stimulus necessary for learning. In addition, place aversion persists for weeks after training (Stinus *et al.*, 2000), and may have a lasting influence on behavior. Learned aversive cues may also impact drug-taking behavior, as demonstrated by evidence that withdrawal-associated stimuli can prime opioid self-administration in dependent animals (Hellemans *et al.*, 2006; Kenny *et al.*, 2006), likely as a means of escaping the noxious experience. Moreover, the role of aversive learning in drug-seeking behavior may also have significant clinical relevance (O'Brien *et al.*, 1975, 1998).

III. Glutamate Systems and Opioid Dependence

Functional ionotropic glutamate receptors are required for the full development and expression of dependence. In particular, it has been shown that acute or chronic administration of NMDA receptor antagonists attenuates physical withdrawal symptoms. This has proven to be a reliable outcome as NMDA receptor blockade inhibits withdrawal in opioid-dependent mice (McLemore *et al.*, 1997), guinea pigs (Tanganelli *et al.*, 1991), rats (Trujillo and Akil, 1991), and humans (Bisaga *et al.*, 2001). Moreover, in rodent models, NMDA antagonists also suppress the conditioned aversive properties (i.e., CPA) of naloxone-precipitated withdrawal (Higgins *et al.*, 1992).

The NMDA receptor has complex signaling properties and is a potent modulator of cellular adaptability (Dingledine *et al.*, 1999; Kohr, 2006). The NMDA receptor is a tetrameric heteromer composed of the essential NMDA-NR1 subunit (NR1), of which there are eight splice variants. Along with NR1, glutamate-responsive NMDA receptors require some combination of NMDA-NR2 subunits (NR2), of which there are four subtypes (NR2A–D). Critical characteristics of the NMDA receptor include a voltage-dependent Mg^{2+} blockade requiring cellular depolarization for channel activation and high permeability to Ca^{2+} . In addition, NMDA receptor activation can modulate numerous intracellular signaling processes including protein kinase activity (Haddad, 2005), protein transport (Shi *et al.*, 1999), transcription factor activity, and gene expression (Yoneda *et al.*, 2001), as well as epigenetic events (Lubin *et al.*, 2008). These properties of NMDA receptor activation may have profound effects on cellular and behavioral plasticity (Tsien, 2000), such as long-term

potentiation (LTP) and long-term depression (LTD) (Kullmann *et al.*, 2000), in addition to whole-animal learning and memory (Shapiro and Eichenbaum, 1999; Walker and Davis, 2002), processes that play a role in opioid dependence.

IV. The Central Nucleus and Dependence

Despite the significant relationship between NMDA receptors and opioid dependence, a brain-map linking sites of functional receptor expression to specific opioid withdrawal behaviors is at best rudimentary. Given that brain motivational systems are a critical substrate of opioid plasticity, NMDA receptors in nuclei such as the CeA are likely to play an important role in dependence. The central amygdala is a critical component of neural motivation pathways that play a role in endocrine (Schulkin *et al.*, 1994) and autonomic function (Brody, 1988), fluid (McKinley *et al.*, 2001) and energy balance (Glass *et al.*, 2000), as well as behavioral (Davis, 1998) and affective (Davis, 1989) processes. Many of these activities are known to be modulated by glutamate and opioids. In order to better appreciate the role of the CeA glutamate system in opioid dependence, a brief overview of its place within the larger context of brain circuitry subserving motivated behavior is illustrated in Fig. 8.1. The basic neurochemical content of the CeA will be outlined below.

The CeA has a diverse complement of glutamate afferents that arise from areas of brain motivational systems. These include limbic cortices (McDonald, 1998), the thalamus (Turner and Herkenham, 1991), and the basolateral nucleus of the amygdala (BLA) (Pitkanen *et al.*, 1997). The CeA is notable for its abundant expression of glutamate receptors (Petralia and Wenthold, 1992; Petralia *et al.*, 1994; Sato *et al.*, 1993). In particular, neurons in the CeA express the NR1 gene (Sato *et al.*, 1995) and protein (Petralia *et al.*, 1994), as well as NMDA ligand-binding sites (Monaghan and Cotman, 1985).

In addition to glutamate, CeA neurons have a rich array of signaling molecules. Many central amygdala neurons express the enzyme glutamic acid decarboxylase 67 kDa, which is responsible for synthesizing the inhibitory transmitter γ -aminobutyric acid (GABA) (Carta *et al.*, 2008). In addition to GABA, there are also endogenous CeA neurons and/or axons from extrinsic sources that express a variety of neuroactive peptides as well as receptors for these modulators. These include corticotropin-releasing factor (Beyer *et al.*, 1988; Swanson *et al.*, 1983), components of the renin–angiotensin system (Brown and Gray, 1988; Brownfield *et al.*, 1982; Lavoie *et al.*, 2004), neuropeptide Y (Gray *et al.*, 1986; Heilig *et al.*, 1993), orexin (Baldo *et al.*, 2003), oxytocin (Lee *et al.*, 2005; Roozendaal *et al.*, 1993), and vasopressin (Francis *et al.*, 2002; Roozendaal *et al.*, 1993). In addition, the CeA is also innervated by catecholamine releasing axonal varicosities (Asan, 1998) and contains neurons that express adrenergic receptors (Glass *et al.*, 2002).

Among the peptide systems active in the central amygdala, the opioids are notably abundant. Enkephalin (Cassell *et al.*, 1986; Petrovich *et al.*, 2000; Wray and Hoffman, 1983), dynorphin (Fallon and Leslie, 1986; Reyes *et al.*, 2008; Zardetto-Smith *et al.*, 1988), and β -endorphin (Gray *et al.*, 1984) are each present in the CeA. Moreover, opioid receptors, including the μ OR, are expressed in the central amygdala (Mansour *et al.*, 1988, 1994). Deciphering the synaptic organization and functional relationships between NMDA

receptors and opioid receptors is a critical issue in understanding of central amygdala function and its relationship to opioid dependence.

V. The Synaptic Relationship Between NMDA and μ -Opioid Receptors in the CeA

The cellular relationship between NMDA receptors and μ ORs in the CeA has only been inferred by the results of electrophysiological studies, and is a matter of contention (Zhu and Pan, 2004, 2005). Because of its high spatial resolution, immunoelectron microscopy employing gold and peroxidase markers is a valuable tool for examining the fine structural location of functionally interacting proteins in areas of brain motivational pathways (Gracy and Pickel, 1995; Van Bockstaele *et al.*, 2000). Using immunoelectron microscopic analysis, the NR1 subunit was shown to be highly enriched in postsynaptic (i.e., dendrites) sites of central amygdala neurons (Fig. 8.2A and B) (Glass *et al.*, 2009). Labeled dendritic profiles were typically small to intermediate in size (0.5–1 μ m cross-sectional area). NR1 was frequently present in near intracellular endomembranous organelles, reflecting sites of protein storage and trafficking. However, this protein was also present on the plasma membrane, the primary location of functional receptors. Furthermore, many of these dendritic profiles were contacted by axon terminals forming asymmetric excitatory-type synapses, typical of those made by glutamatergic axon terminals (Peters *et al.*, 1991). In summary, NR1 was mainly localized to dendritic (i.e., postsynaptic) structures where it was frequently present in vesicular organelles linked to protein transport, as well as the extrasynaptic and synaptic plasma membrane contacted by excitatory-type axon terminals.

In terms of its subcellular location, the μ OR was found near intracellular membranous organelles and the plasma membrane, a pattern similar to that of NR1. However, relative to the glutamate receptor subunit, the μ OR had a more heterogeneous distribution in neuronal compartments. Although frequently present in somata and dendrites, there were also many instances of μ OR expressing axons and axon terminals (for details see Glass *et al.*, 2009).

There were numerous instances of neuronal profiles in the CeA that expressed labeling for both NR1 and the μ OR. Despite the prominent dendritic distribution of NR1 and the mixed dendritic and axonal localization for the μ OR, dual labeling for these proteins was most commonly found in dendrites (Fig. 8.2C and D). Like the single-labeled NR1 and μ OR containing dendrites, structures that expressed immunoreactivity for both proteins were small to medium in size. Labeling for each protein was present near vesicular organelles characteristic of those involved in protein transport, and was also found on the surface membrane. Synapses formed on these dual labeled dendrites were typically of the asymmetric excitatory kind. In sum, these results indicate that NMDA receptors and μ ORs are strategically positioned for postsynaptic comodulation of glutamate signaling in dendrites of CeA neurons.

VI. Deletion of Postsynaptic NR1 in Central Amygdala Neurons Attenuates Opioid Withdrawal-Induced Place Aversion

Establishing relationships between functional postsynaptic NMDA receptor expression and opioid dependence is not feasible by traditional pharmacological approaches. Currently available NMDA receptor antagonists cannot discriminate between neuronal dendrites, axon terminals, or other ultrastructural compartments. In addition, interpreting neuropharmacological studies involving NMDA receptor antagonists is problematic given that central amygdala blockade of NMDA receptors can have rewarding or aversive properties, depending on the particular agent used (Watanabe *et al.*, 2002), as well as other effects likely to confound performance of learned behaviors including alterations in motor function (Andrzejewski *et al.*, 2004).

To examine the relationship between opioid dependence and functional postsynaptic central amygdala NMDA receptors a spatial-temporal gene deletion strategy employing Cre-lox technology was used. The NR1 subunit was deleted by local CeA microinjection of a neurotropic replication deficient recombinant rAAV that expressed a fusion protein of the enzyme Cre recombinase (Cre) and a reporter, GFP, termed “rAAV-GFP-Cre” (South *et al.*, 2003). Injections were made in adult male transgenic loxP knockin mice that have strategically placed loxP sites in the NR1 gene (i.e., “floxed NR1” (fNR1) mice) flanking exons that encode for the four membrane domains and the C-terminus (South *et al.*, 2003).

Direct microinjection of rAAV-GFP-Cre into the CeA of adult fNR1 mice resulted in recombination specifically in neurons (Glass *et al.*, 2008). Moreover, there was a significant reduction in NR1 expression in the target area (Fig. 8.3). This reduction occurred in somata and dendritic profiles (i.e., postsynaptic). There were no concomitant reductions of presynaptic NR1 or somatodendritic NR2 immunolabeling, and no effects on local cell number or cellular morphology (Glass *et al.*, 2008).

Bilateral knockout of the NR1 gene in the CeA did not produce obvious basal behavioral deficits. In particular, deletion of CeA NR1 did not impact locomotor activity, body weight, sensory-motor coordination, thermal nociception, or somatosensation (Glass *et al.*, 2008).

In regard to opioid dependence, CeA NR1 knockout mice were chronically exposed to morphine by subcutaneously implanted morphine pellets and then administered naloxone to precipitate withdrawal. The CeA NR1 knockouts did not differ from control animals with respect to somatic signs, such as escape-jumping and wet-dog shakes, or autonomic symptoms, notably diarrhea and weight loss (Fig. 8.4A). Contrary to what was found with physical symptoms, central amygdala NR1 gene deletion did impair naloxone withdrawal-induced place aversion (Fig. 8.4B). Therefore, functional CeA NMDA receptors were not necessary for the induction of many major physical withdrawal symptoms, but were required for the production of a learned withdrawal-induced negative affective state.

The finding that CeA NR1 deletion impaired withdrawal-induced CPA is consistent with prior reports that NMDA receptor activity in the CeA plays an important role in aversive learning and memory (Goosens and Maren, 2003). The precise role of the central amygdala

in emotional learning has been controversial. Traditionally, the CeA has been considered less as a site of learning and more as a modulator of other areas that encode new associations, however, emerging evidence contradicts this view. For example, recent findings have shown that the CeA is required for the normal acquisition and consolidation of conditioned fear (Rabinak and Maren, 2008; Wilensky *et al.*, 2006). These neurological results are supported by neurophysiological data. It has been reported that neurons in the CeA exhibit a form of cellular plasticity (i.e., LTP) that occurs in established neuroanatomical substrates of learning and memory, such as the hippocampus and lateral amygdala (Samson and Paré, 2005). Moreover, CeA LTP is also sensitive to withdrawal from drugs of abuse in an NMDA receptor-dependent manner (Pollandt *et al.*, 2006).

VII. Does the CeA Selectively Participate in the Conditioned Aversive Properties of Opioid Withdrawal?

The finding that CeA NR1 deletion selectively interferes with CPA has interesting parallels with other reports in the literature. For example, in opioid-dependent animals, withdrawal induced by CeA microinjection of an opioid antagonist preferentially produces CPA (Stinus *et al.*, 1990). In addition, it has been shown that lesioning the ventral noradrenergic bundle, which provides an important source of norepinephrine containing axons in the CeA and related areas of the extended amygdala, inhibits opioid withdrawal place aversive (Delfs *et al.*, 2000). The latter effect is paralleled by the ability of CeA administered alpha-2-adrenergic agonists to inhibit only affective signs of withdrawal (Taylor *et al.*, 1998). Based on this evidence, one may conclude that the CeA has a privileged role in mediating the conditioned emotional properties of withdrawal.

The notion that the CeA has a special role in affective learning processes related to dependence, however, may be too simplistic. For example, it has been reported that blockade of non-NMDA type glutamate receptors in the CeA of dependent animals reduces somatic signs of opioid withdrawal (Taylor *et al.*, 1998). This result is similar to other findings in the literature showing that blockade of CeA CRF receptors also inhibits physical symptoms of withdrawal from opioids (McNally and Akil, 2002).

It appears that manipulating particular receptor systems in the CeA can produce distinct outcomes with respect to the acute physical and psychological features of opioid withdrawal. One reasonable explanation for these complex outcomes is that particular manifestations of dependence may be coded by distinct neurochemical signals acting within circumscribed central amygdala circuits. One hypothetical scheme detailing how such a system may be organized is illustrated in Fig. 8.5.

VIII. Conclusion

Both NMDA receptors and μ ORs are strategically positioned for the comodulation of excitatory postsynaptic signaling in CeA neurons. Moreover, genetic deletion of postsynaptic NMDA receptors impairs the conditioned aversive, but not the physical features of opioid withdrawal. Because conditioned cues may promote behaviors to avoid or escape their associated adverse state, NMDA receptor activity in the CeA may be essential

in complex neural processes that engage relief-seeking behaviors, like drug taking (Koob, 2009).

The intriguing speculation that the neural substrates of particular behavioral features of dependence may involve differing synaptic organizational arrangements within central amygdala circuitry will require further exploration. This hypothesis can be addressed by, among other techniques, multilabeling immunoelectron microscopic ultrastructural analysis. This can be combined with application of spatial–temporal gene deletion methodology similar to that described in this chapter, as well as a development of this approach that incorporates phenotype-specific promoters to manipulate gene expression in selected neurochemical populations (Jasnow *et al.*, 2009; Oh *et al.*, 2009). Such efforts should elucidate the synaptic and molecular bases of the many manifestations of dependence, knowledge that may be critical in developing the next generation of neurobiological tools to manage the long-term consequences of opioid abuse. Moreover, this approach also has implications for other psychiatric disorders involving aversive learning, such as anxiety disorders.

References

- Allen GV, Cechetto DF. Neurotensin in the lateral hypothalamic area: Origin and function. *Neuroscience*. 1995; 69:533–544. [PubMed: 8552247]
- Andrzejewski ME, Sadeghian K, Kelley AE. Central amygdalar and dorsal striatal NMDA receptor involvement in instrumental learning and spontaneous behavior. *Behav Neurosci*. 2004; 118:715–729. [PubMed: 15301599]
- Asan E. The catecholaminergic innervation of the rat amygdala. *Adv Anat Embryol Cell Biol*. 1998; 142:1–118. [PubMed: 9586282]
- Baldo BA, Daniel RA, Berridge CW, Kelley AE. Overlapping distributions of orexin/hypocretin- and dopamine-beta-hydroxylase immunoreactive fibers in rat brain regions mediating arousal, motivation, and stress. *J Comp Neurol*. 2003; 464:220–237. [PubMed: 12898614]
- Beyer HS, Matta SG, Sharp BM. Regulation of the messenger ribonucleic acid for corticotropin-releasing factor in the paraventricular nucleus and other brain sites of the rat. *Endocrinology*. 1988; 123:2117–2123. [PubMed: 3262055]
- Bisaga A, Comer SD, Ward AS, Popik P, Kleber HD, Fischman MW. The NMDA antagonist memantine attenuates the expression of opioid physical dependence in humans. *Psychopharmacology*. 2001; 157:1–10. [PubMed: 11512037]
- Brody MJ. Central nervous system and mechanisms of hypertension. *Clin Physiol Biochem*. 1988; 6:230–239. [PubMed: 3060299]
- Brown MR, Gray TS. Peptide injections into the amygdala of conscious rats: Effects on blood pressure, heart rate, and plasma catecholamines. *Reg Peptides*. 1988; 21:95–106.
- Brownfield MS, Reid IA, Ganten D, Ganong WF. Differential distribution of immunoreactive angiotensin and angiotensin-converting enzyme in rat brain. *Neuroscience*. 1982; 7:1759–1769. [PubMed: 6289182]
- Brownstein MJ. A brief history of opiates, opioid peptides, and opioid receptors. *Proc Natl Acad Sci*. 1993; 90:5391–5393. [PubMed: 8390660]
- Buccafusco JJ. Participation of different brain regions in the anti-narcotic withdrawal action of clonidine in the dependent rat. *Brain Res*. 1990; 513:8–14. [PubMed: 2350687]
- Carta AR, Moreno CC, Cadoni C, Tronci E, Di Chiara G. Long-term increase in GAD67 mRNA expression in the central amygdala of rats sensitized by drugs and stress. *Eur J Neurosci*. 2008; 27:1220–1230. [PubMed: 18312583]

- Cassell MD, Gray TS, Kiss JZ. Neuronal architecture in the rat central nucleus of the amygdala: A cytological, hodological, and immunocytochemical study. *J Comp Neurol.* 1986; 246:478–499. [PubMed: 2422231]
- Christie MJ. Cellular neuroadaptations to chronic opioids: Tolerance, withdrawal and addiction. *Br J Pharmacol.* 2008; 154:384–396. [PubMed: 18414400]
- Cunningham CL, Gremel CM, Groblewski PA. Drug-induced conditioned place preference and aversion in mice. *Nat Protocols.* 2006; 1:1662–1670. [PubMed: 17487149]
- Davis M. Neural systems involved in fear-potentiated startle. *Ann NY Acad Sci.* 1989; 563:165–183. [PubMed: 2570545]
- Davis M. Are different parts of the extended amygdala involved in fear versus anxiety? *Biol Psychiatry.* 1998; 44:1239–1247. [PubMed: 9861467]
- Delfs JM, Zhu Y, Druhan JP, Aston-Jones G. Noradrenaline in the ventral forebrain is critical for opiate withdrawal-induced aversion. *Nature.* 2000; 403:430–434. [PubMed: 10667795]
- Dingledine R, Borges K, Bowie D, Traynelis SF. The glutamate receptor ion channels. *Pharmacol Rev.* 1999; 51:7–61. [PubMed: 10049997]
- Eisenberg RM, Sparber SB. Changes in plasma corticosterone levels as a measure of acute dependence upon levorphanol in rats. *J Pharmacol Exp Ther.* 1979; 211:364–369. [PubMed: 574160]
- Fallon JH, Leslie FM. Distribution of dynorphin and enkephalin peptides in the rat brain. *J Comp Neurol.* 1986; 249:293–336. [PubMed: 2874159]
- Francis DD, Young LJ, Meaney MJ, Insel TR. Naturally occurring differences in maternal care are associated with the expression of oxytocin and vasopressin (V1a) receptors: Gender differences. *J Neuroendocrinol.* 2002; 14:349–353. [PubMed: 12000539]
- Glass MJ, Billington CJ, Levine AS. Naltrexone administered to central nucleus of amygdala or PVN: Neural dissociation of diet and energy. *Am J Physiol.* 2000; 279:R86–R92.
- Glass MJ, Colago EE, Pickel VM. Alpha-2A-adrenergic receptors are present on neurons in the central nucleus of the amygdala that project to the dorsal vagal complex in the rat. *Synapse.* 2002; 46:258–268. [PubMed: 12373741]
- Glass MJ, Hegarty DM, Oselkin M, Quimson L, South SM, Xu Q, Pickel VM, Inturrisi CE. Conditional deletion of the NMDA-NR1 receptor subunit gene in the central nucleus of the amygdala inhibits naloxone-induced conditioned place aversion in morphine dependent mice. *Exp Neurol.* 2008; 213:57–70. [PubMed: 18614169]
- Glass MJ, Vanyo L, Quimson L, Pickel VM. Ultrastructural relationship between NMDA-NR1 and mu-opioid receptor in the mouse central nucleus of the amygdala. *Neuroscience.* 2009; 163:857–867. [PubMed: 19607886]
- Gonzalez ML, Milanes MV, Martinez-Pinero MG, Marin MT, Vargas ML. Effects of intracerebroventricular clonidine on the hypothalamic noradrenaline and plasma corticosterone levels of opiate naive rats and after naloxone-induced withdrawal. *Brain Res.* 1994; 647:199–203. [PubMed: 7922496]
- Goosens KA, Maren S. Pretraining NMDA receptor blockade in the basolateral complex, but not the central nucleus, of the amygdala prevents savings of conditional fear. *Behav Neurosci.* 2003; 117:738–750. [PubMed: 12931959]
- Gracy KN, Pickel VM. Comparative ultrastructural localization of the NMDAR1 glutamate receptor in the rat basolateral amygdala and bed nucleus of the stria terminalis. *J Comp Neurol.* 1995; 362:71–85. [PubMed: 8576429]
- Gracy KN, Dankiewicz LA, Koob GF. Opiate withdrawal-induced FOS immunoreactivity in the rat extended amygdala parallels the development of conditioned place aversion. *Neuropsychopharmacology.* 2001; 24:152–160. [PubMed: 11120397]
- Gray TS, Cassell MD, Kiss JZ. Distribution of pro-opiomelanocortin-derived peptides and enkephalins in the rat central nucleus of the amygdala. *Brain Res.* 1984; 306:354–358. [PubMed: 6087978]
- Gray TS, O'Donohue TL, Magnuson DJ. Neuropeptide Y innervation of amygdaloid and hypothalamic neurons that project to the dorsal vagal complex in rat. *Peptides.* 1986; 7:341–349. [PubMed: 2426686]

- Gray TS, Carney ME, Magnuson DJ. Direct projections from the central amygdaloid nucleus to the hypothalamic paraventricular nucleus: Possible role in stress-induced adrenocorticotropin release. *Neuroendocrinology*. 1989; 50:433–446. [PubMed: 2554178]
- Haddad JJ. N-methyl-D-aspartate (NMDA) and the regulation of mitogen-activated protein kinase (MAPK) signaling pathways: A revolving neurochemical axis for therapeutic intervention? *Prog Neurobiol*. 2005; 77:252–282. [PubMed: 16343729]
- Hamlin A, Buller KM, Day TA, Osborne PB. Peripheral withdrawal recruits distinct central nuclei in morphine-dependent rats. *Neuropharmacology*. 2001; 41:574–581. [PubMed: 11587712]
- Heilig M, McLeod S, Brot M, Heinrichs SC, Menzaghi F, Koob GF, Britton KT. Anxiolytic-like action of neuropeptide Y: Mediation by Y1 receptors in amygdala, and dissociation from food intake effects. *Neuropsychopharmacology*. 1993; 8:357–363. [PubMed: 8099792]
- Hellems KG, Dickinson A, Everitt BJ. Motivational control of heroin seeking by conditioned stimuli associated with withdrawal and heroin taking by rats. *Behav Neurosci*. 2006; 120:103–114. [PubMed: 16492121]
- Higgins GA, Nguyen P, Sellers EM. The NMDA antagonist dizocilpine (MK801) attenuates motivational as well as somatic aspects of naloxone precipitated opioid withdrawal. *Life Sci*. 1992; 50:PL167–PL172. [PubMed: 1533700]
- Hyman SE, Malenka RC, Nestler EJ. Neural mechanisms of addiction: The role of reward-related learning and memory. *Annu Rev Neurosci*. 2006; 29:565–598. [PubMed: 16776597]
- Inturrisi CE. Clinical pharmacology of opioids for pain. *Clin J Pain*. 2002; 18(4 Suppl):S3–S13. [PubMed: 12479250]
- Jasnow AM, Rainnie DG, Maguschak KA, Chhatwal JP, Ressler KJ. Construction of cell-type specific promoter lentiviruses for optically guiding electro-physiological recordings and for targeted gene delivery. *Methods Mol Biol*. 2009; 515:199–213. [PubMed: 19378132]
- Kawasaki Y, Jin C, Suemaru K, Kawasaki H, Shibata K, Choshi T, Hibino S, Gomita Y, Araki H. Effect of glutamate receptor antagonists on place aversion induced by naloxone in single-dose morphine-treated rats. *Br J Pharmacol*. 2005; 145:751–757. [PubMed: 15880144]
- Kenny PJ, Chen SA, Kitamura O, Markou A, Koob GF. Conditioned withdrawal drives heroin consumption and decreases reward sensitivity. *J Neurosci*. 2006; 26:5894–5900. [PubMed: 16738231]
- Kest B, Palmese CA, Hopkins E, Adler M, Juni A, Mogil JS. Naloxone-precipitated withdrawal jumping in 11 inbred mouse strains: Evidence for common genetic mechanisms in acute and chronic morphine physical dependence. *Neuroscience*. 2002; 115:463–469. [PubMed: 12421612]
- Kohr G. NMDA receptor function: Subunit composition versus spatial distribution. *Cell Tissue Res*. 2006; 326:439–446. [PubMed: 16862427]
- Koob GF. Neurobiological substrates for the dark side of compulsivity in addiction. *Neuropharmacology*. 2009; 56(Suppl 1):18–31. [PubMed: 18725236]
- Korostynski M, Piechota M, Kaminska D, Solecki W, Przewlocki R. Morphine effects on striatal transcriptome in mice. *Genome Biol*. 2007; 8:R128. [PubMed: 17598886]
- Kreek MJ. Role of a functional human gene polymorphism in stress responsivity and addictions. *Clin Pharmacol Ther*. 2008; 83:615–618. [PubMed: 18323858]
- Kullmann DM, Asztely F, Walker MC. The role of mammalian ionotropic receptors in synaptic plasticity: LTP, LTD, and epilepsy. *Cell Mol Life Sci*. 2000; 57:1551–1561. [PubMed: 11092450]
- Lavoie JL, Cassell MD, Gross KW, Sigmund CD. Localization of renin expressing cells in the brain, by use of a REN-eGFP transgenic model. *Physiol Genomics*. 2004; 16:240–246. [PubMed: 14625376]
- LeDoux JE. Emotion circuits in the brain. *Ann Rev Neurosci*. 2000; 23:155–184. [PubMed: 10845062]
- Lee PR, Brady DL, Shapiro RA, Dorsa DM, Koenig JI. Social interaction deficits caused by chronic phencyclidine administration are reversed by oxytocin. *Neuropsychopharmacology*. 2005; 30:1883–1894. [PubMed: 15798779]
- Lubin FD, Roth TL, Sweatt JD. Epigenetic regulation of BDNF gene transcription in the consolidation of fear memory. *J Neurosci*. 2008; 28:10576–10586. [PubMed: 18923034]

- Maldonado R, Stinus L, Gold LH, Koob GF. Role of different brain structures in the expression of the physical morphine withdrawal syndrome. *J Pharmacol Exp Ther.* 1992; 261:669–677. [PubMed: 1578378]
- Mansour A, Khachaturian H, Lewis ME, Akil H, Watson SJ. Anatomy of CNS opioid receptors. *Trends Neurosci.* 1988; 11:308–314. [PubMed: 2465635]
- Mansour A, Fox CA, Thompson RC, Akil H, Watson SJ. mu-Opioid receptor mRNA expression in the rat CNS: Comparison to mu-receptor binding. *Brain Res.* 1994; 643:245–265. [PubMed: 8032920]
- Marcilhac A, Siaud P. Identification of projections from the central nucleus of the amygdala to the paraventricular nucleus of the hypothalamus which are immunoreactive for corticotrophin-releasing hormone in the rat. *Exp Physiol.* 1997; 82:273–281. [PubMed: 9129941]
- Martin WR, Wikler A, Eades CG, Pescor FT. Tolerance to and physical dependence on morphine in rats. *Psychopharmacologia.* 1963; 4:247–260. [PubMed: 14048545]
- Matthes HWD, Maldonado R, Simonin F, Valverde O, Slowe S, Kitchen I, Befort K, Dierich A, Le Meur M, Dolle P, Tzavara E, Hanoune J, et al. Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the mu-opioid-receptor gene. *Nature.* 1996; 383:819–823. [PubMed: 8893006]
- McDonald AJ. Cortical pathways to the mammalian amygdala. *Prog Neurobiol.* 1998; 55:257–332. [PubMed: 9643556]
- McKinley MJ, Allen AM, Mathai ML, May C, McAllen RM, Oldfield BJ, Weisinger RS. Brain angiotensin and body fluid homeostasis. *Jpn J Physiol.* 2001; 51:281–289. [PubMed: 11492952]
- McLemore GL, Kest B, Inturrisi CE. The effects of LY293558, an AMPA receptor antagonist, on acute and chronic morphine dependence. *Brain Res.* 1997; 778:120–126. [PubMed: 9462883]
- McNally GP, Akil H. Role of corticotropin-releasing hormone in the amygdala and bed nucleus of the stria terminalis in the behavioral, pain modulatory, and endocrine consequences of opiate withdrawal. *Neuroscience.* 2002; 112:605–617. [PubMed: 12074902]
- Monaghan DT, Cotman CW. Distribution of N-methyl-D-aspartate-sensitive L-[3H]glutamate-binding sites in rat brain. *J Neurosci.* 1985; 5:2909–2919. [PubMed: 2865341]
- Mucha RF. Is the motivational effect of opiate withdrawal reflected by common somatic indices of precipitated withdrawal? A place conditioning study in the rat. *Brain Res.* 1987; 418:214–220. [PubMed: 3676713]
- O'Brien CP. Evidence-based treatments of addiction. *Philos Trans R Soc Lond B Biol Sci.* 2008; 363:3277–3286. [PubMed: 18640914]
- O'Brien CP, O'Brien TJ, Mintz J, Brady JP. Conditioning of narcotic abstinence symptoms in human subjects. *Drug Alcohol Depend.* 1975; 1:115–123. [PubMed: 1235105]
- O'Brien CP, Childress AR, Ehrman R, Robbins SJ. Conditioning factors in drug abuse: Can they explain compulsion? *J Psychopharmacol.* 1998; 12:15–22. [PubMed: 9584964]
- Oh MS, Hong SJ, Huh Y, Kim KS. Expression of transgenes in midbrain dopamine neurons using the tyrosine hydroxylase promoter. *Gene Ther.* 2009; 16:437–440. [PubMed: 18800154]
- Peters, A.; Palay, SL.; Webster, H. *The Fine Structure of the Nervous System.* Oxford University Press; New York: 1991.
- Petralia RS, Wenthold RJ. Light and electron immunocytochemical localization of AMPA-selective glutamate receptors in the rat brain. *J Comp Neurol.* 1992; 318:329–354. [PubMed: 1374769]
- Petralia RS, Yokotani N, Wenthold RJ. Light and electron microscope distribution of the NMDA receptor subunit NMDAR1 in the rat nervous system using a selective anti-peptide antibody. *J Neurosci.* 1994; 14:667–696. [PubMed: 8301357]
- Petrovich GD, Scicli AP, Thompson RF, Swanson LW. Associative fear conditioning of enkephalin mRNA levels in central amygdalar neurons. *Behav Neurosci.* 2000; 114:681–686. [PubMed: 10959526]
- Phelps EA, LeDoux JE. Contributions of the amygdala to emotion processing: From animal models to human behavior. *Neuron.* 2005; 48:175–187. [PubMed: 16242399]
- Pitkanen A, Savander V, LeDoux JE. Organization of intra-amygdaloid circuitries in the rat: An emerging framework for understanding functions of the amygdala. *Trends Neurosci.* 1997; 20:517–523. [PubMed: 9364666]

- Pollandt S, Liu J, Orozco-Cabal L, Grigoriadis DE, Vale WW, Gallagher JP, Shinnick-Gallagher P. Cocaine withdrawal enhances long-term potentiation induced by corticotropin-releasing factor at central amygdala glutamatergic synapses via CRF, NMDA receptors and PKA. *Eur J Neurosci*. 2006; 24:1733–1743. [PubMed: 17004937]
- Rabinak CA, Maren S. Associative structure of fear memory after basolateral amygdala lesions in rats. *Behav Neurosci*. 2008; 122:1284–1294. [PubMed: 19045948]
- Reyes BA, Drolet G, Van Bockstaele EJ. Dynorphin and stress-related peptides in rat locus coeruleus: Contribution of amygdalar efferents. *J Comp Neurol*. 2008; 508:663–675. [PubMed: 18381633]
- Roosendaal B, Schoorlemmer GH, Koolhaas JM, Bohus B. Cardiac, neuroendocrine, and behavioral effects of central amygdaloid vasopressinergic and oxytocinergic mechanisms under stress-free conditions in rats. *Brain Res Bull*. 1993; 32:573–579. [PubMed: 8221155]
- Samson RD, Paré D. Activity-dependent synaptic plasticity in the central nucleus of the amygdala. *J Neurosci*. 2005; 25:1847–1855. [PubMed: 15716421]
- Sato K, Kiyama H, Tohyama M. The differential expression patterns of messenger RNAs encoding non-N-methyl-D-aspartate glutamate receptor subunits (GluR1-4) in the rat brain. *Neuroscience*. 1993; 52:515–539. [PubMed: 8450957]
- Sato K, Mick G, Kiyama H, Tohyama M. Expression patterns of a glutamate-binding protein in the rat central nervous system: Comparison with N-methyl-D-aspartate receptor subunit 1 in rat. *Neuroscience*. 1995; 64:459–475. [PubMed: 7700533]
- Scarborough, J. The opium poppy in hellenistic and roman medicine. In: Porter, R.; Teich, M., editors. *Drugs and narcotics in history*. Cambridge University Press; New York: 1995. p. 4-23.
- Schmued L, Phermsangngam P, Lee H, Thio S, Chen E, Truong P, Colton E, Fallon J. Collateralization and GAD immunoreactivity of descending pallidal efferents. *Brain Res*. 1989; 487:131–142. [PubMed: 2752280]
- Schulkin J, McEwen BS, Gold PW. Allostasis, amygdala, and anticipatory angst. *Neurosci Biobehav Rev*. 1994; 18:385–396. [PubMed: 7984356]
- Schulteis G, Yackey M, Risbrough V, Koob GF. Anxiogenic-like effects of spontaneous and naloxone-precipitated opiate withdrawal in the elevated plus-maze. *Pharmacol Biochem Behav*. 1998; 60:727–731. [PubMed: 9678657]
- Shapiro ML, Eichenbaum H. Hippocampus as a memory map: Synaptic plasticity and memory encoding by hippocampal neurons. *Hippocampus*. 1999; 9:365–384. [PubMed: 10495019]
- Shi SH, Hayashi Y, Petralia RS, Zaman SH, Wenthold RJ, Svoboda K, Malinow R. Rapid spine delivery and redistribution of AMPA receptors after synaptic NMDA receptor activation. *Science*. 1999; 284:1811–1816. [PubMed: 10364548]
- South SM, Kohno T, Kaspar BK, Hegarty D, Vissel B, Drake CT, Ohata M, Jenab S, Sailer AW, Malkmus S, Masuyama T, Horner P, et al. A conditional deletion of the NR1 subunit of the NMDA receptor in adult spinal cord dorsal horn reduces NMDA currents and injury-induced pain. *J Neurosci*. 2003; 23:5031–5040. [PubMed: 12832526]
- Stinus L, Le Moal M, Koob GF. Nucleus accumbens and amygdala are possible substrates for the aversive stimulus effects of opiate withdrawal. *Neuroscience*. 1990; 37:767–773. [PubMed: 2247222]
- Stinus L, Caille S, Koob GF. Opiate withdrawal-induced place aversion lasts for up to 16 weeks. *Psychopharmacology*. 2000; 149:115–120. [PubMed: 10805605]
- Stinus L, Cador M, Zorrilla EP, Koob GF. Buprenorphine and a CRF1 antagonist block the acquisition of opiate withdrawal-induced conditioned place aversion in rats. *Neuropsychopharmacology*. 2005; 30:90–98. [PubMed: 15138444]
- Swanson, LW. *Brain Maps: Structure of the Rat Brain*. Elsevier; Amsterdam: 1992.
- Swanson LW. Cerebral hemisphere regulation of motivated behavior. *Brain Res*. 2000; 886:113–164. [PubMed: 11119693]
- Swanson LW, Sawchenko PE, Rivier J, Vale WW. Organization of ovine corticotropin-releasing factor immunoreactive cells and fibers in the rat brain: An immunohistochemical study. *Neuroendocrinology*. 1983; 36:165–186. [PubMed: 6601247]
- Tanganelli S, Antonelli T, Morari M, Bianchi C, Beani L. Glutamate antagonists prevent morphine withdrawal in mice and guinea pigs. *Neurosci Lett*. 1991; 122:270–272. [PubMed: 2027527]

- Taylor JR, Punch LR, Elsworth JD. A comparison of the effects of clonidine and CNQX infusion into the locus coeruleus and the amygdala on naloxone-precipitated opiate withdrawal in the rat. *Psychopharmacology*. 1998; 138:133–142. [PubMed: 9718282]
- Trujillo KA, Akil H. Inhibition of morphine tolerance and dependence by the NMDA receptor antagonist MK-801. *Science*. 1991; 251:85–87. [PubMed: 1824728]
- Tsien JZ. Linking Hebb's coincidence-detection to memory formation. *Curr Opin Neurobiol*. 2000; 10:266–273. [PubMed: 10753792]
- Turner BH, Herkenham M. Thalamoamygdaloid projections in the rat: A test of the amygdala's role in sensory processing. *J Comp Neurol*. 1991; 313:295–325. [PubMed: 1765584]
- Van Bockstaele EJ, Saunders A, Commons KG, Liu XB, Peoples J. Evidence for coexistence of enkephalin and glutamate in axon terminals and cellular sites for functional interactions of their receptors in the rat locus coeruleus. *J Comp Neurol*. 2000; 417:103–114. [PubMed: 10660891]
- Walker DL, Davis M. The role of amygdala glutamate receptors in fear learning, fear-potentiated startle, and extinction. *Pharmacol Biochem Behav*. 2002; 71:379–392. [PubMed: 11830172]
- Wallace DM, Magnuson DJ, Gray TS. The amygdalo-brainstem pathway: Selective innervation of dopaminergic, noradrenergic and adrenergic cells in the rat. *Neurosci Lett*. 1989; 97:252–258. [PubMed: 2717061]
- Watanabe T, Nakagawa T, Yamamoto R, Maeda A, Minami M, Satoh M. Involvement of glutamate receptors within the central nucleus of the amygdala in naloxone-precipitated withdrawal-induced conditioned place aversion in rats. *Jpn J Pharmacol*. 2002; 88:399–406. [PubMed: 12046982]
- Way EL, Loh HH, Shen FH. Simultaneous quantitative assessment of morphine tolerance and physical dependence. *J Pharmacol Exp Ther*. 1969; 167:1–8. [PubMed: 5814495]
- Weaver M, Schnoll S. Abuse liability in opioid therapy for pain treatment in patients with an addiction history. *Clin J Pain*. 2002; 18(4 Suppl):S61–S69. [PubMed: 12479255]
- Wilensky AE, Schafe GE, Kristensen MP, LeDoux JE. Rethinking the fear circuit: The central nucleus of the amygdala is required for the acquisition, consolidation, and expression of Pavlovian fear conditioning. *J Neurosci*. 2006; 26:12387–12396. [PubMed: 17135400]
- Williams JT, Christie MJ, Manzoni O. Cellular and synaptic adaptations mediating opioid dependence. *Physiol Rev*. 2001; 81:299–343. [PubMed: 11152760]
- Wray S, Hoffman GE. Organization and interrelationship of neuropeptides in the central amygdaloid nucleus of the rat. *Peptides*. 1983; 4:525–541. [PubMed: 6196761]
- Yoneda Y, Kuramoto N, Kitayama T, Hinoi E. Consolidation of transient ionotropic glutamate signals through nuclear transcription factors in the brain. *Prog Neurobiol*. 2001; 63:697–719. [PubMed: 11165002]
- Zahm DS, Jensen SL, Williams ES, Martin JR. Direct comparison of projections from the central amygdaloid region and nucleus accumbens shell. *Eur J Neurosci*. 1999; 11:1119–1126. [PubMed: 10103108]
- Zardetto-Smith AM, Moga MM, Magnuson DJ, Gray TS. Lateral hypothalamic dynorphinergic efferents to the amygdala and brainstem in the rat. *Peptides*. 1988; 9:1121–1127. [PubMed: 2469062]
- Zhu W, Pan ZZ. Synaptic properties and postsynaptic opioid effects in rat central amygdala neurons. *Neuroscience*. 2004; 127:871–879. [PubMed: 15312899]
- Zhu W, Pan ZZ. μ -opioid-mediated inhibition of glutamate synaptic transmission in rat central amygdala neurons. *Neuroscience*. 2005; 133:97–103. [PubMed: 15893634]

Figure 8.1.

The organization of brain motivational pathways involved in opioid dependence. Brain motivational systems are composed of a highly complex network of extero- and interoceptive processing systems, integrative memory systems, as well as hypothalamic and brainstem endocrine/autonomic and motor activators that play essential roles in maintaining homeostasis (Swanson, 2000). The CeA is a critical coordinator of relevant functional inputs and outputs within this circuitry, and may play a role in coupling homeostatic need with emotional valence and immediate survival behaviors, as well as more slowly developing learned emotional responses to those challenging conditions. A highly schematic representation of neural pathways involved in motivational processes is presented as a series of illustrated coronal brain sections. The CeA and its outputs are indicated in gray, while specific targets are highlighted by red fill. Relevant CeA inputs are indicated by differently colored dashed arrows. These include glutamate afferents (green) from areas of limbic

cortices (McDonald, 1998), the thalamus (Turner and Herkenham, 1991), and the BLA. The CeA is also innervated by catecholaminergic neurons (black), including dopaminergic neurons from the mesolimbic system and noradrenergic neurons from the medulla (Asan, 1998). Neuropeptides (blue) from these and other brain areas are also expressed by CeA inputs (see text for description). This array of afferents provides the CeA with a complex set of signals about current internal and external stimuli, as well as representations in memory, that are critical in organizing behavioral responses. Both NMDA-type glutamate and μ ORs are expressed within the central amygdala. Critical outputs of the central amygdala include areas of the extended amygdala involved in learned emotional processes such as the bed nucleus of the stria terminalis (BNST; Zahm *et al.*, 1999), hypothalamic systems involved in endocrine (paraventricular nucleus of the hypothalamus [PVN]) and behavioral (lateral hypothalamic area [LHA]) responses (Allen and Cechetto, 1995; Gray *et al.*, 1989; Marcilhac and Siaud 1997), midbrain areas involved in nociception (periaqueductal gray [PAG]), reward (ventral tegmental area [VTA]), and/or motor (substantia nigra [SN]) function (Schmued *et al.*, 1989; Zahm *et al.*, 1999), as well as medullary nuclei (nucleus of the solitary tract [NTS], ventrolateral medulla [VLM]) that mediate cardiovascular, respiratory, and/or gastrointestinal processes (Glass *et al.*, 2002; Wallace *et al.*, 1989). Drawings are adapted from the brain atlas of Swanson (Swanson, 1992).

Figure 8.2.

Ultrastructural distribution of the NMDA receptor and its relationship to the μ OR in the central amygdala. (A) A dendritic profile (NR-1-d) expressed diffuse immunoperoxidase reaction product for NR1. This profile exhibited a spine neck (sp-n) and was contacted by an unlabeled axon terminal (ut-1) that did not appear to form a synapse. (B) When labeled by either immunoperoxidase (ABC) or immunogold (Gold) secondary markers, the majority of NR1-labeled processes were dendrites. (C) A dendritic profile (NR-1 and μ OR-d) expressed diffuse immunoperoxidase reaction product for NR1 and immunogold labeling (small arrows) for the μ OR. This dual labeled profile received distinct asymmetric excitatory-type synapses (arrow heads) from two unlabeled axon terminals (ut-1 and ut-2). (D) Of all dual labeled neuronal profiles, the majority were dendrites. This was the case when NR1 was labeled by immunoperoxidase (ABC) and the μ OR by immunogold (Gold) markers, and when secondary antisera were reversed (i.e., NR1 labeled by gold and the μ OR by peroxidase). Scale bars: 0.5 μ m. See Glass *et al.* (2009) for details.

Figure 8.3.

Postsynaptic NR1 deletion in central amygdala neurons. (A) Unilateral microinjection of rAAV-GFP-Cre (indicated by arrow) in the CeA produced a localized knockout of NR1 as shown by *in situ* hybridization. (B) Gene deletion corresponded to expression of the GFP reporter as seen in a serial section. GFP expressing neurons can be seen at a higher magnification in the inset. (C, D) Higher magnification views of the CeA as seen in Fig. 8.3A. Note the significantly diminished NR1 expression in the injected hemisphere (C) compared to the uninjected (D) side. (E) Compared to unilateral CeA injection of the control GFP vector, injection of Cre produced significant reductions in NR1 labeling in somatodendritic sites in the injected compared to the uninjected hemisphere as measured by immunoelectron microscopy (NR1 immuno). This corresponded to reductions in NR1 gene

expression (NR1 *in situ*) in the CeA (Glass *et al.*, 2008). * $p < 0.05$ compared to GFP. Scale bars: 1 mm.

Figure 8.4.

Postsynaptic deletion of NR1 in central amygdala neurons and opioid dependence. (A) Separate groups of fNR1 mice, including those given no intracranial injection (No-inj), or bilaterally microinjected with either rAAV-GFP (GFP) or rAAV-GFP-Cre (Cre), were chronically administered morphine by subcutaneous implantation of a morphine pellet (25 mg), which was replaced by a fresh pellet every fourth day as needed. Dependence was determined by visual observations of physical withdrawal signs including diarrhea, wet-dog shakes, and escape jumping after an acute injection of naloxone (1 mg/kg, i.p.). There were no differences between any of the groups in the number of somatic (wet-dog shakes + jumping) or autonomic (diarrhea; not shown) symptoms. (B). Place aversion training took place in an apparatus consisting of a two-chamber box, each with distinct tactile and visual cues, that was inserted into an automated activity monitor. After measuring baseline selection, during which time animals were allowed to freely explore each chamber, animals began place aversion training. On alternate training days morphine dependent mice were injected with saline (0.9%, i.p.) or naloxone (1 mg/kg, i.p.) and then restricted to the respective chamber for 30 min. On the test day, subjects were allowed to freely explore both chambers for 30 min as during baseline. The difference in time spent in the naloxone-paired chamber during the testing and preconditioning phases served as the measure of place aversion. In distinction to physical withdrawal, there were significant between-group differences in CPA. Unlike animals in both control groups, mice bilaterally microinjected with Cre in the CeA did not spend less time in the withdrawal-paired chamber, indicating that CeA NR1 knockout interfered with a conditioned place aversion in response to opioid withdrawal. Reductions in NR1 mRNA were seen selectively in the animals injected with rAAV-GFP-Cre as previously reported (Glass *et al.*, 2008).

Figure 8.5.

Schematic representation illustrating hypothetical model of central amygdala synaptic coding of physical and psychological signals linked with opioid withdrawal. One group of CeA neurons may express non-NMDA type (i.e., AMPA, kainate), glutamate (dark green stars), and CRF (blue stars) receptors, and receive preferential input from CRF expressing neurons (blue arrow). The physical withdrawal pathway may be engaged by an upstream opioid receptor-dependent pathway, presumably expressing glutamate or CRF. This system would be expected to mediate immediate opioid withdrawal symptoms. The other group (light gray shading) of CeA neurons may express NMDA (pale green stars), mu-opioid (yellow star), and noradrenergic (black star) receptors, and receive input from noradrenergic

afferents (black arrow), particularly from areas of the medulla (NTS, VLM), as well as opioid peptides (not shown). This system would be expected to have a slow onset and require consolidation and long-lasting synaptic integration. Glutamate (green arrow) would be expected to activate both systems, however, whether segregation of function is mediated by separate input sources (i.e., source of glutamate), or by distinct processing capacities (i.e., glutamate receptor types) of their targets is uncertain. The role of CRF (Stinus *et al.*, 2005) and non-NMDA (Kawasaki *et al.*, 2005) receptors may not be limited to immediate unconditional responses, and an alternative model would incorporate these signaling systems into both immediate physical and affective/learning processing streams.

Table 8.1

In small rodents, opioid withdrawal symptoms can be categorized into distinct functional groupings, including somatic, autonomic, endocrine, and affective (Buccafusco, 1990; Gonzalvez *et al.*, 1994; Martin *et al.*, 1963; Mucha, 1987)

Somatic	Escape-jumping, wet-dog shakes
Autonomic	Diarrhea, hypertension
Endocrine	ACTH and Cort release
Affective	Acute aversion
Learning	Conditioned place aversion/avoidance

Only some of the most prominent examples are listed for each category.

ACTH: adrenocorticotrophic hormone; Cort: corticosterone