CHAPTER 15

μ-Opioid Receptors, Placebo Map, Descending Systems, and Cingulate-Mediated Control of Vocalization and Pain

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Chapter contents

Efforts to control pain, anxiety, depression, and chronic stress syndromes increasingly seek to target the cingulate cortex as one of the privileged sites of "top–down regulation" of emotional motor systems. Its role in anticipation, the expectation of pain control via placebo events and hypnosedation are discussed throughout this section of the book in great detail. Although critical information processing including access to emotional memories, valenced objects and contexts, assessment of behavioral outcomes and links to internal states depend on cingulate cortex, its role in implementing control of emotional motor systems is equally important. Indeed, cingulate cortex has one of the most extensive cortical and subcortical projection systems to regulate emotional output of the cerebral cortex as outlined below. The pivotal location of μ-opioid receptors (MOR) in anterior cingulate and midcingulate cortices (ACC, MCC, respectively) assures a role for opioids in mediating these massive output systems and is likely why the opiate placebo is such a powerful event in medical practice and pharmaceutical interventions.

Classical views of limbic cortex emphasized the role of cingulate cortex in emotion and family-related behavior (MacLean, 1990, 1993). Although the MacLean-Papez model (MacLean, 1954) did not have specific sites for control of emotional motor systems, MacLean's later formulation of the thalamocingulate division of the triune brain identifies maternal–infant interactions as a key example of family-related behavior and its mediation by vocalization, the separation cry, and even pain associated with separation. This view led Paul MacLean and neuroscientists of the 1970s and 1980s to explore the mechanisms of vocalization through the periaqueductal gray (PAG; Müller-Preuss & Jürgens, 1976; Jürgens & Pratt, 1979; MacLean & Newman, 1988; Newman, 1988). Exploration of this system and cingulate driving of emotionally coded vocalizations such as conspecific cooing and cackling within the monkey troop and interactions between infant and mother represented the first and pivotal consideration of specific mechanisms by which cingulate cortex regulates emotional motor outputs.

The 1970s were a time of other important research findings in cingulate function and control of descending systems. Talairach *et al.* (1973) reported that electrical stimulation of cingulate cortex (MCC) evoked complex movements such as lip puckering, entire limb and bilateral limb movements, and kneading of the fingers. One patient reported a playful gesture as to communicate an invitation to play. Geier *et al.* (1977) reported that, during a seizure which included cingulate cortex (pregenual ACC), one patient dreamed that she was playing with a friend. These are emotional expressions of affiliation not simple movements. Lip puckering, for example, is not a calibrated movement of oral skeletal muscle but rather a kissing gesture. Although the mechanisms of such movements were not understood at that time, these are the ground breaking observations that still lead today the investigation of mechanisms of cingulate regulation of emotional expression. Finally, Hiller *et al.* (1973) reported that the potent opiate agonist etorphine had the highest binding in limbic cortex including ACC. Much research in the last two decades seeks to elaborate on this early approach to studying specific outputs of cingulate cortex in terms of autonomic and skeletomotor control of emotional motor systems and the mechanisms of control of these systems by opiate compounds.

Descending control: Beyond the PAG

It is an interesting fact that the cingulate projection to the PAG was the first to be intensively explored as a mechanism of vocalization and emotional expression, while a growing body of human imaging evidence also implicates the PAG in descending control of pain as discussed here and in other chapters. The specific role of the PAG in such studies, however, has not been definitively shown because of the lack of spatial resolution in current fMRI. The involvement of this particular structure is often a matter of speculation from activity in larger activation sites in the midbrain which can include the raphe nuclei, locus coeruleus, and other adjacent nuclei that also have a role in regulating pain processing at the spinal level. Although the specific structures generating these responses are not known, the projections from parts of cingulate cortex to many nuclei in this region have been shown in experimental animals to be quite extensive. One goal of this chapter is to put the specific cytological and connection organization into a framework that can be applied to human imaging research. It also seeks to expand our views of pain control to the parabrachial (PB) nucleus, disinhibition of the PAG by cingulate cortex rather than only excitation and to explore a growing body of evidence in rodents that cingulate cortex is pivotal to a descending noxious facilatory system (DNFS). Thus, views of cingulate-mediated descending control systems for regulating pain processing must be greatly expanded beyond classical views of PAG functions.

Enumerating descending projections from ACC and MCC

The anterior part of the cingulate gyrus probably has a more extensive descending projection system than any other cortical region. To make this point, the following is a list of target structures identified in rodents and primates that are reviewed in this chapter and/or discussed in other chapters. The numbers in parentheses refer to the relevant chapter in this volume that discusses the particular connection or a citation. The list is

arranged from rostral to caudal and does not include cortical projections:

1 Nucleus accumbens and caudate nucleus (Chapter 28)
2 Anterior, midline, mediodorsal and intralaminar thalamic nuclei (Chapter 4)
3 Claustrum (Van Hoesen *et al.*, 1993)
4 Amygdala (Chapters 6, 9, 15)
5 Hypothalamus (Chapters 10, 15, 22)
6 Periaqueductal gray (Chapter 15)
7 Parabrachial nucleus (Chapter 15)
8 Locus coeruleus (Chapter 22)
9 Pontine nuclei (Glickstein *et al.*, 1985)
10 Red nucleus (Van Hoesen *et al.*, 1993)
11 Raphe nuclei (Chapter 15)
12 Facial motor nucleus (Morecraft *et al.*, 1996)
13 Subnucleus reticularis dorsalis (Chapter 15)
14 Nucleus of the tractus solitarius and dorsal motor nucleus of the vagus (Neafsey *et al.*, 1993)
15 Spinal cord (Dum & Strick, 1993)

Goals of This Chapter

"Top–down regulation" of emotional motor systems is critically dependent on anticipation and the expectation of pain control and on engaging these systems to induce sedation with hypnosis. This chapter seeks to detail the specific mechanisms of MOR control of particular neuron populations and circuits. Co-registration of active placebo sites to the four-region model provides a synthesis of mechanisms, whereby opiate drugs and medically induced placebos regulate descending systems that originate mainly in ACC and MCC. The specific aims include the following:

1 Demonstrate the distribution of MOR binding in human and links to emotion activations and emotional expression.
2 Describe the distribution of MOR binding in monkey with slide autoradiography and link this binding to classes of output neurons in layers III and V that contribute to descending control systems.
3 Link MOR to afferent and intrinsic systems and their linkage to stimulation of G-proteins with autoradiography in the rat.
4 Enumerate the MOR-driven intracortical cascade circuit that directly controls the descending noxious inhibitory system via the PAG.
5 Evaluate the distribution of placebo events in a cingulate flat map to relate different mechanisms of placebo generation.
6 Characterize the structure and connections of the "cingulate vocalization area" and assess its role in emotional motor control.
7 Evaluate links between cingulate cortex and the descending noxious inhibitory system primarily through the PAG and PB nuclei in relation to pain control.
8 Identify cingulate participants in the descending noxious facilatory system and enumerate a model of cingulate descending control of pain and chronic stress circuits.
9 Summarize the major output systems for autonomic and skeletomotor control in the form of a tripartite efferent system from the sACC, pACC and aMCC.

μ-Opioid Receptors

Binding and agonist stimulation

Opioid compounds widely control the nociceptive and pain systems from the nociceptor itself and extending to the medial-limbic pain system. It has been known for over three decades that limbic cortex including the anterior cingulate gyrus has a high level of opioid binding (Hiller *et al.*, 1973) and a number of *in vivo* imaging studies of opioid receptor distribution and activation report a predominance of activity in this region. Studies of the volume of distribution of diprenorphine binding, a non-selective opioid ligand, is high in ACC and moderate in MCC (Sadzot *et al.*, 1991; Vogt *et al.*, 1995; Fig. 15.1). A factoral analysis of this binding showed that ACC and MCC are among the four regions that explained 45% of the variance in Factor 1 for diprenorphine binding (Baumgärtner *et al.*, 2006). Carfentanil is selective for MOR and also has a high density in ACC, although localization resolution was poor in early studies (Frost *et al.*, 1990). Thus, it is likely that, although diprenorphine is a non-selective ligand, the high density of diprenorphine binding in ACC is dominated by MOR binding.

Our study of the volume of distribution of diprenorphine binding in human cingulate gyrus was done in the context of the four-region neurobiological model (Vogt *et al.*, 1995) and it showed the highest binding in ACC including the subgenual ACC, moderate levels in MCC with none detectable in the cingulate sulcal areas, and patchy binding in dorsal posterior cingulate cortex (dPCC) with almost none in the ventral PCC. This study raised questions about the lack of opioid regulation of the sulcal cingulate motor areas as considered below in the monkey brain, the extent to which agonist

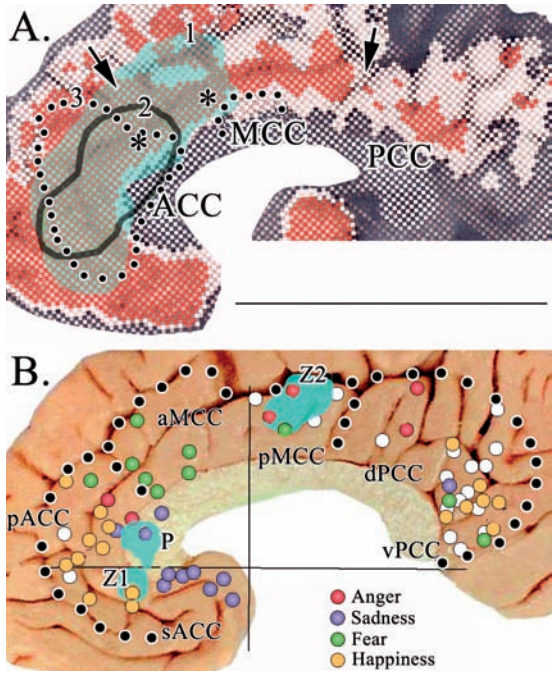

Fig. 15.1 Opioid receptor binding and placebos in the context of the four-region model. (A) Volume of distribution for diprenorphine plotted on the medial surface with the borders of pACC and ACC/MCC marked with arrows and elevations in nociceptive activity in a thermal pain study (Vogt *et al.*, 1995, 1996; *). Three levels of binding include high (red pixels), moderate (white pixels) and low (gray pixels). MOR stimulation sites reported by Adler *et al.* (1997; solid black line), Schlaepfer *et al.* (1998; blue), and Wagner *et al.* (2001; dotted lines). (B) Peak voxels activated in studies of simple emotions (Vogt *et al.*, 2003), the opiate-placebo site reported by Petrovic *et al.* (2002; P) and MOR and affective regulation of binding by Zubieta *et al.* (2001, Z2; 2003, Z1).

stimulation of MOR alters activity in the same regions and in different layers, and whether or not there was a resolution problem for binding in the cingulate motor areas and the vPCC.

The distribution of nociceptive activations has also been reviewed in terms of the four-region neurobiological model and they heavily co-localize to MCC (Vogt *et al.*, 2003; Vogt, 2005). Thus, we can now directly link nociceptive activity in relation to MOR in this framework. Figure 15.1 shows the locations of our two activation sites generated during noxious thermal stimulation of the hand (Vogt *et al.*, 1996); one site is in the caudal part of pACC and the other in aMCC (asterisks in the figure). Another study of nociceptive responses showed elevated activity in pACC associated with focusing on the unpleasantness of a noxious stimulus (Kulkarni *et al.*, 2005). The four-region model predicts that nociceptive activity associated with emotion is generated in ACC and that activity associated with premotor planning, anticipation and assessment of outcomes is performed in aMCC. If this is the case, opiate drugs could actually enhance activity in nociceptive cingulate cortex to trigger descending pain control circuits.

Stimulation of MOR produces an interesting paradox. Although MOR couples to Gi/Go proteins that enhance potassium-channel functions and other inhibitory actions as discussed below, stimulation of pACC and aMCC with opiate drugs does not reduce activity in these regions, but rather, stimulate this region as does hypnosis prior to generating analgesia (Chapter 17) and motor cortex stimulation during analgesic responses (Chapter 20). It appears that the analgesic effects mediated by ACC and aMCC are associated with activation of these regions including excitation via opiate drugs. One explanation of these analgesic phenomena is that each, including MOR stimulation, drives the descending noxious inhibitory system rather than directly blocking intracingulate emotion.

Fentanyl, its rapidly acting congener remifentanil, and hydromorphone activate mainly pACC and aMCC. Figure 15.1 shows the location of the main elevation in activity in these regions generated in studies by Adler *et al.* (1997), Schlaepfer *et al.* (1998), and Wagner *et al.*, (2001). Leppä *et al.* (2006) showed a wider pattern of cingulate activations during remifentanil administration and one that more closely reflects the pattern of binding except for that in sACC were activity was not observed. Clearly, MOR stimulation activates pACC and aMCC and this is associated with the autonomic and analgesic properties of these drugs as predicted by the four-region model. It is of interest, however, that the binding and activation patterns do not mirror each other.

It is striking that even though MOR binding is high throughout ACC, the primary region of activation is pACC with little or no activation of sACC. The sACC has high MOR binding capacity and activity associated with negatively valenced emotions yet is not activated in these studies (Vogt, 2005). Leppä did not activate sACC with remifentanil but only pACC and only a small and probably non-significant increase in regional cerebral blood flow (rCBF) in sACC was shown by Petrovic *et al.* (2002). This confirms that MOR activation does not significantly impact the region of negative emotion but rather areas associated with positively valenced experiences (happiness; Fig. 15.1). Since remifentanil is selective for MOR, the explanation for non-selective opioid receptor binding by diprenorphine in sACC cannot be due to δ-opioid receptors in sACC. The affinity state and/or G-protein coupling of MOR in human sACC may be quite different from those throughout the remainder of the cingulate gyrus. Thus, the generally elevated mood generated by compounds that stimulate the MOR and increase activity in pACC during hypnosedation

(Chapter 17) may be due to activation of regions generally associated with positive emotions.

These observations raise many questions of the links between MOR stimulation and emotional responses and many questions remain about the density and distribution of MOR binding in the primate cingulate gyrus. Is it true that the cingulate motor areas in the depths of the cingulate sulcus are without MOR regulation as suggested in human studies? What layers express the most pronounced binding and what are the links between peaks in laminar binding and descending control systems? Surely, it is the link between receptor binding and particular populations of neurons that provides for the analgesic effects of opiate compounds and, to the extent that conscious mechanisms generated in the cingulate gyrus mediate the analgesic actions of these drugs, the MOR link with particular populations of projection neurons is pivotal information that can only be determined with experimental animals.

MOR localization in monkey

Higher resolution localization of MOR binding, including that to particular layers and areas, is possible in monkey with section autoradiography than is the case for human *in vivo* methods. Here, we consider the distribution of [^{3}H]Tyr-D-Ala-Gly-MePhe-Gly-ol (DAMGO; MOR agonist) binding in an adult cynomolgus monkey according to concentrations and blockers to determine non-specific binding as previously reported (Vogt L. J. *et al.*, 2001). Figure 15.2 shows DAMGO binding in the cingulate gyrus that was calibrated to microscales to assess concentrations of binding (nCi/mg protein). The levels were sampled through the entire gyrus and oriented to a flat map of another adult cynomolgus monkey (Vogt *et al.*, 2005). The section autoradiographs were thresholded for imaging to the layer with highest binding, i.e., layers III–V.

Peak levels of DAMGO binding are in ACC including areas 25, 32, and 24 as well as in aMCC as shown for

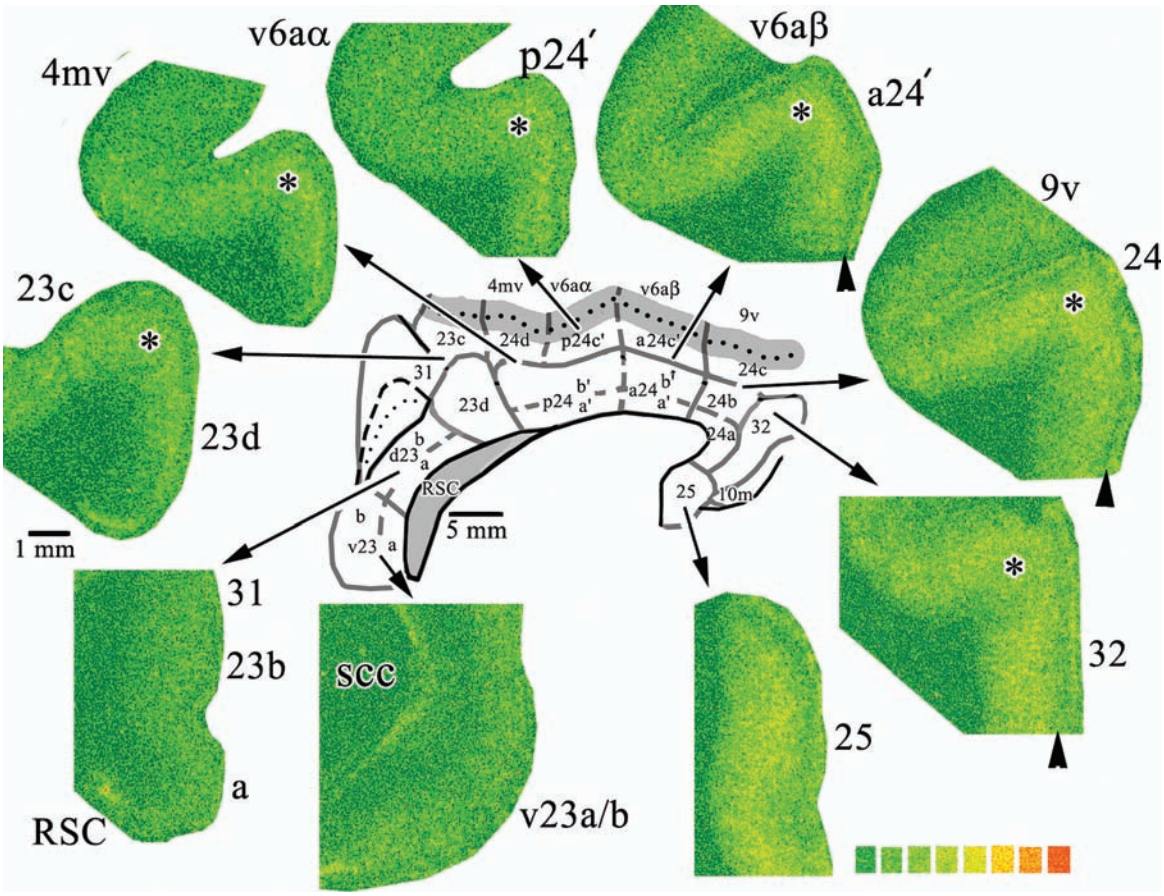

Fig. 15.2 DAMGO binding in monkey in relation to a flat map of the cingulate gyrus with the sulcal fundus marked in gray as is RSC in the callosal sulcus. Highest binding is in areas 25, 32, 24 and a24′. The asterisks identify peak binding in layer III at the apical border between areas 32 and 24b and for each border between the "b/c" divisions. The microscales in this and the next figure represent nCi/mg tissue: 0, 1.3, 2.1, 3.3, 5.3, 8.3, 13.2, 21.2. RSC, retrosplenial cortex; scc, splenium of corpus callosum; dotted line below "31" indicates the fundus of splenial sulcus.

area a24′. DAMGO binding is moderate in pMCC and dPCC and low in vPCC. There also appears to be a small peak in the most medial parts of RSC. Of pivotal importance to regulation of the cingulate premotor areas (CPMA; synonymous with the cingulate motor areas CMA discussed in Chapter 5) by MOR, DAMGO binding is high in the rostral CPMA in areas 24c and a24c′. The high density of binding in the ventral bank of the cingulate sulcus is not matched by a similarly high level of binding in the neocortical motor areas in the dorsal bank of the sulcus (i.e., areas 9v, v6aβ v6aα, 4mv). These patterns on the dorsal and ventral banks of the cingulate sulcus confirm a previous conclusion that the CPMAs do not extend onto the dorsal bank of the cingulate sulcus in the monkey (Vogt *et al.*, 2005); although they do so in the human (Vogt & Vogt, 2003).

An asterisk at the apex of the cingulate gyrus marks the border between the gyral "b" and sulcal "c" divisions in each subregion. Sulcal cortex with the rostral CPMA is located to the left of each asterisk in areas 32, 24, and a24′. Clearly, the rCPMA has a high level of DAMGO binding. Thus, the hypothesis from human studies that highest binding occurs in rostral areas ACC and aMCC is supported, while the expectation of limited direct MOR regulation of the rCPMA is not.

The low-magnification autoradiographs in Figure 15.2 do not provide detailed information about the laminar localization of DAMGO binding and this can be achieved by co-registering the sections used to generate the autoradiographs, and subsequently stained with thionin, to the autoradiograph itself. These co-registered images are shown in Figure 15.3 at a higher magnification and

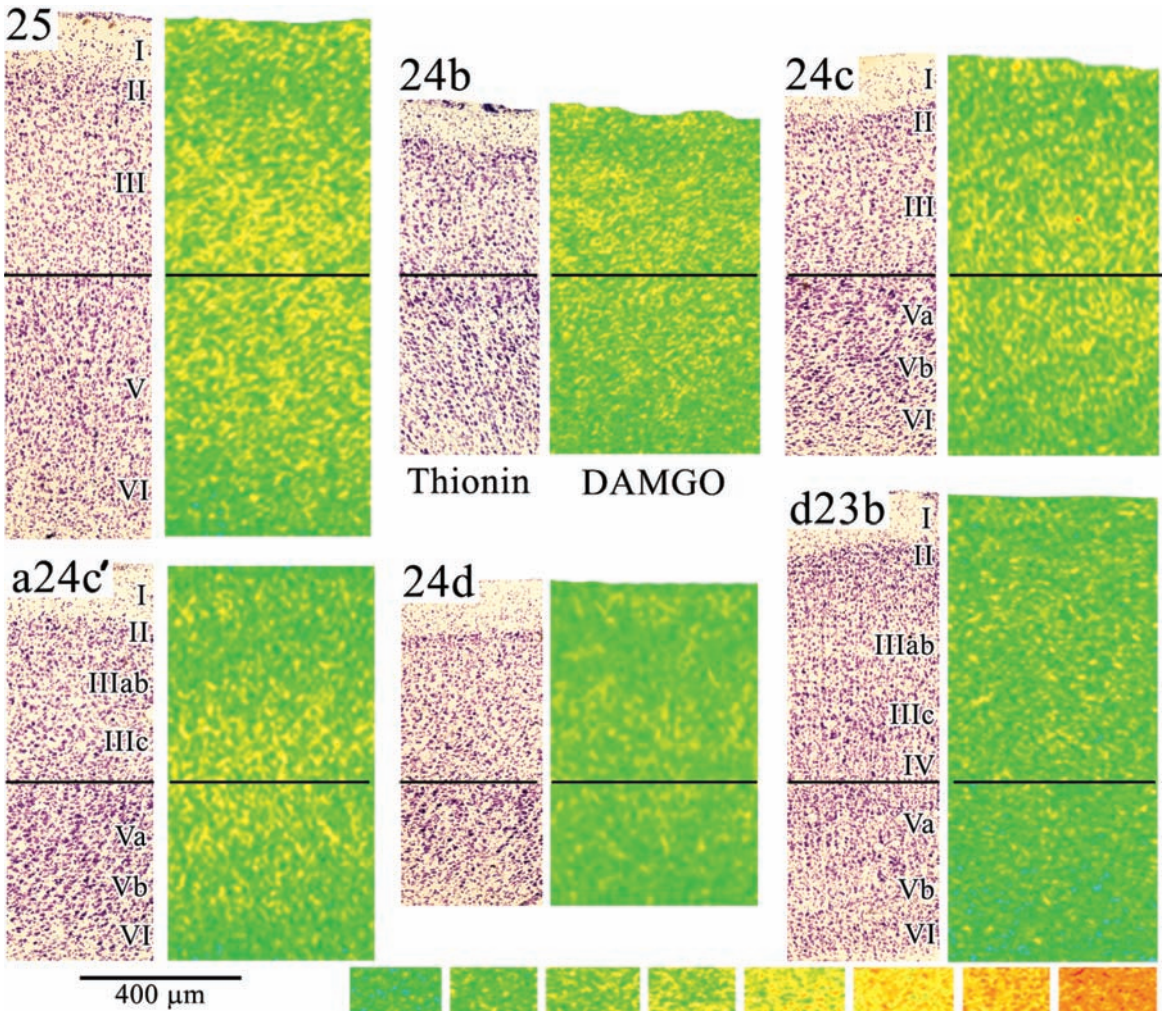

Fig. 15.3 Laminar foci of DAMGO binding in selected areas of monkey cortex demonstrated with thionin-stained sections (left of each pair) co-registered to color-coded binding (right of each pair). All sections are aligned with a black line at the top of layer Va which is always neuron dense in cingulate cortex. Peak binding is in layer III/IIIc in all instances with further significant binding in layer V when binding is high (areas 25–32 not shown 24b, 24c, a24c′) and relatively more in superficial layers where binding is moderate to low (e.g., areas 24d and d23b). Microscales coded as in previous figure.

provide examples of binding in each region of the cingulate gyrus. Highest binding in all instances is in layers III or IIIc depending on the area. When binding is highest in ACC and aMCC, a secondary peak is also in layer Va. Only when overall binding is lower, as in caudal areas 24d and d23b, is there virtually no binding in the deep layers and what DAMGO binding there is, is diffuse in the superficial layers. Thus, there is a rostrocaudal (*y* axis) variation in the laminar expression pattern of DAMGO binding.

MOR regulation of the rostral and caudal CPMAs is quite different in two ways. First, MOR are 2.5 times higher in layer III of the rCPMA (i.e., areas 24c, a24c′) than in layer IIIc the cCPMA (i.e., areas p24c′, 24d, 23c). Secondly, MOR are very high in layers III and Va of the rCPMA, while in the cCPMA, they are mainly in layer IIIc. A laminar hypothesis of MOR regulation of the CPMAs suggests that the rCPMA has regulation of corticocortical functions via layer III binding and potentially direct regulation of corticostriatal outputs from layer Va, while the cCPMA is mainly regulated by a moderate density of MOR in the corticocortical projection layer III and none in deeper layers. Thus, the CPMAs and their efferent systems are differentially regulated by MOR.

MOR neuron localization and G-protein coupling

Cytoarhitectonic analysis co-registered to laminar MOR binding in the monkey is a prelude to neuron localization of receptor binding and a further analysis of transduction mechanisms. Look again at area a24c′ in Figure 15.3 to see that peak binding in layer IIIc is associated with large and deep lying neurons in this layer and note the relatively high density of binding in layer Va with a dense population of large projection neurons. Of course, there are also multipolar interneurons in both of these layers and more than one class of projection neurons are present as well. The latter include multiple cortical projections from layer IIIc and projections to the PAG, stratum and pontine nuclei from layer Va. Nevertheless, the co-registration of binding to cytologically unique layers is the first step to understanding the mechanisms of opiate drug actions in the cingulate gyrus. Localization of binding to particular afferent and efferent projection systems is accomplished with experimental lesions in rodent brain.

Acute removal of a cingulate afferent input or one or more populations of projection neurons, i.e., post-lesion survival times of 1–2 weeks, leads to a loss of receptors expressed on those axons or the somatodendritic membranes of the degenerating neurons. We used undercut lesions to remove all afferent axons (Vogt L. J. *et al.*, 2001). Alternatively, selective lesions of the thalamus with thermocoagulation lesions or the locus coeruleus with the immunotoxin OX7-saporin conjugated to an antibody to dopamine-ß hydroxylase (DBH) have been used to remove subsets of afferent axons to cingulate cortex (Vogt *et al*, 1995; Vogt L. J. *et al.*, 2001) to localize receptors on thalamic and locus coeruleus afferents.

Binding of DAMGO is normally highest in layer I of rat area 24, lowest in layer II and low to moderate in deeper layers with a secondary peak in layer VI. The undercut lesions, an example of which is shown in Figure 15.4, produced more than a 60% reduction of DAMGO binding in layer I demonstrating that the large majority of MOR in this layer are associated with afferent axons. Notice that the secondary peak in layer VI also has reduced DAMGO binding but it is only about half as much suggesting there is a lower proportionate density of axonal MOR in layer VI than in layer I. The rationale for attributing the loss to presynaptic rather than postsynaptic sites has been made in opioid and other receptor systems (serotonin, Crino *et al.*, 1990; muscarinic acetylcholine, Vogt & Burns, 1988).

The sources of presynaptic/axonal MOR appear to arise from two structures. Thalamic nuclei that project to cingulate cortex are the anterior thalamic and midline nuclei which include the anteromedial nucleus with projections to the ACC and MCC (Vogt *et al.*, 1992). The locus coeruleus was removed with immunotoxin lesions and reduced DAMGO binding by 30% in layer I (Vogt L. J. *et al.*, 2001). Note also that the immunotoxin lesions of DBH are specific as there is no change in DAMGO binding in deeper layers including layer VI after such lesions. Finally, about 35% of DAMGO binding in layer I and 60% in deeper layers are associated with intrinsic neurons because this is what remains after undercut lesions that removes all afferents. Additionally, intrinsic neurons have been destroyed with the excitotoxin ibotenic acid and this demonstrates a similar proportion of binding to intrinsic cortical neurons (Vogt *et al.*, 1995).

Transduction of MOR function is via Gi/Go proteins and activation of these proteins has been assessed with similar ablation methods and correlated with DAMGO binding in the same experimental groups of animals. Links between receptor and G-protein densities suggest differences in transduction mechanisms and could explain why MOR stimulation with opiate compounds discussed earlier activate pACC but not sACC; there is a greater density of G-proteins in the former subregion than in sACC, where the numbers of G-proteins are lower and coupling is weaker. Transduction has been studied in rodents (Vogt L. J. *et al.*, 2001) and monkey (Sim-Selly *et al.*, 1999) and an example of the rat findings along with experimental lesions is shown in Figure 15.4. The DAMGO-stimulated [^{35}S]guanylyl-5′-O-(γ-Thio)-triphosphate ([^{35}S]GTPγS) labeling is highest in layers I

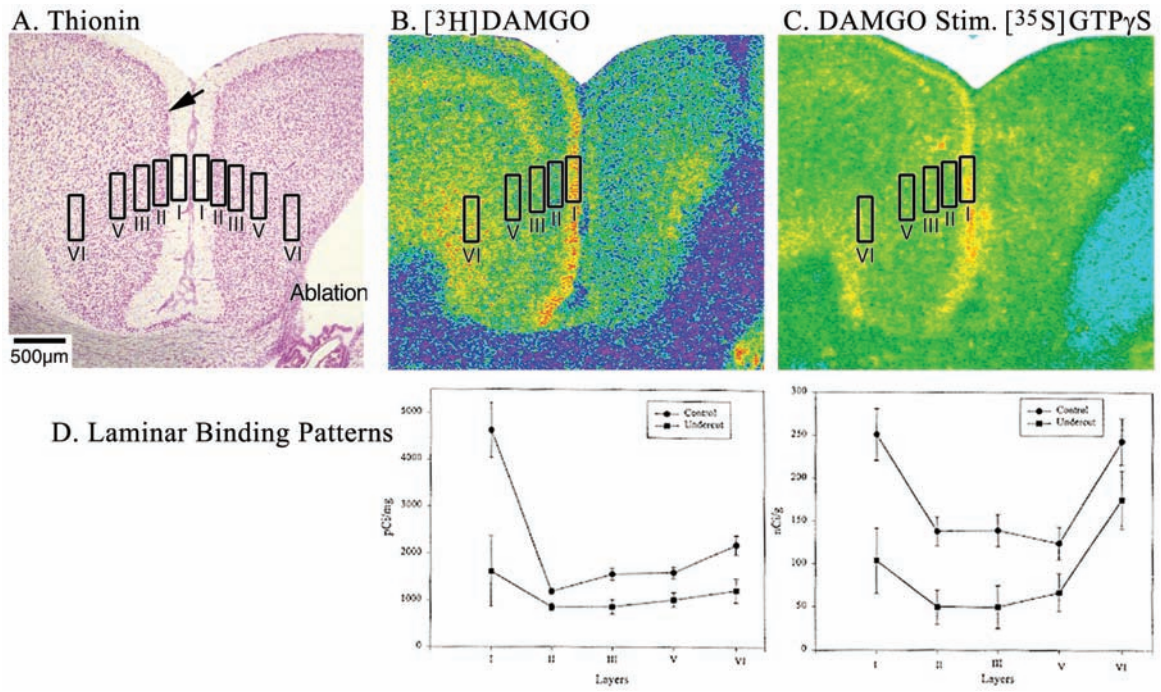

Fig. 15.4 DAMGO binding and stimulated [^{35}S]GTPγS binding in rodent with experimental localization of binding following an undercut lesion (right; 2-week survival) to remove all afferent axons. Laminar sampling sites are shown with the rectangles and the arrow in (A) is the border between AGm laterally and area 24b medially. There is a layer I peak in DAMGO binding in control cases in (B) and for DAMGO stimulated [^{35}S]GTPγS activity. The graphs below each autoradiograph show the mean±SEM for all cases in control and undercut cases. There is a massive reduction in binding and stimulation in the ablated hemispheres with greatest proportionate changes for each in layer I. Although binding is significantly changed in layer VI, G-protein stimulation did not differ in this layer. For further experimental and statistical details see Vogt L. J. *et al.* (2001).

and VI, which is similar to the pattern for DAMGO binding although the layer VI level is lower in the binding. This is likely because there is a higher level of intrinsic neuron G-protein stimulation in layer VI and a limited expression on axon terminals of extrinsic origin. This can be seen with the pattern of stimulation following undercut lesions in Figure 15.4C. The binding in layer VI is almost the same as the controls in layer VI, while in layers I–III (particularly layer I), there is a very significant reduction in stimulation following removal of afferent axons (Fig. 15.4D). Thus, the relative proportions of DAMGO-evoked, G-protein stimulation differs in each layer and is linked to the relative proportion of neurons and afferent axons expressing MOR.

MOR links to human emotion

One method of linking emotion and MOR binding is by plotting both on a flat map of human cingulate cortex and comparing their overlap in the four-region model as in Figure 15.1. As already noted, there is a striking correlation between high levels of MOR binding and activations during sadness in sACC, however, MOR stimulation studies generally failed to activate this subregion. Another strategy for evaluating these links is with a paradigm in which sustained sadness was generated by cued recall of an autobiographical sad event and evaluating [^{11}C]carfentanil availability, presumably due to an increase in MOR stimulation, and statistical analysis without reference to regions of interest (Zubieta *et al.*, 2003). Under these conditions, MOR availability was reduced mainly in the superior part of sACC shown in Figure 15.1 (Z1) at the junction of the greatest number of activations reported during sad events (purple dots in the figure).

During sustained noxious stimulation, a high level of negative affect is expected, and Zubieta *et al.* (2001) evaluated [^{11}C]carfentanil binding during such stimulation. In this paradigm, binding was negatively correlated (i.e., activated by occupied MOR) in pMCC as shown in Figure 15.1 (Z2). In addition, although this response depended largely on four outliers and changes were lost by using an ample distribution for ACC, emotion associated with sustained pain and measured with carfentanil volume of distribution appear to be generated in MCC in a manner similar to expectations for other nociceptive responses in this region rather than

emotion *per se* (Vogt, 2005). Indeed, the emotional response in this paradigm appears to have a limited influence on MOR binding which, instead, may be more closely related to motor output planning, anticipation of pain and orienting the body to the noxious stimuli as discussed in detail in the previous chapter. Nevertheless, it is of interest that MOR ligands can be used to probe the functions of MCC where MOR density is moderate and not as dense as in ACC. It is not surprising that this paradigm generated a MOR response outside of sACC when the specific functions of pMCC are understood in terms of the specific contributions of this region to pain processing and it is realized that sACC has no known role in acute pain processing.

Axons of the MITN and locus coeruleus express MOR

Experimental studies in rats demonstrate two afferent systems in ACC that are regulated by MOR expressed directly on afferent axons as discussed above. One of these is the locus coeruleus (LC) as demonstrated with the immunotoxin OX7-saporin. A second source is the midline, mediodorsal and intralaminar thalamic nuclei (MITN) that are long known to express MOR (Hiller *et al.*, 1973) and on their axon terminals in cingulate cortex (Vogt *et al.*, 1995). In view of the dual locations of MORs, some of the most important actions of these receptors are likely in blocking transmission though stress-linked activation of the LC and nociceptive processing transmitted via the MITN. As both MOR systems are located on axon terminals and couple to Gi/Go inhibitory proteins that inhibit adenylyl cylase activity, they block entry of calcium into the terminal to make both systems quiescent as discussed further below.

MOR inhibit neuronal activity yet elevate rCBF

MOR stimulation in various experimental preparations generally show that these receptors inhibit adenylyl cyclase activity via Gi/Go proteins, block calcium channels in axon terminals and somata, as well as open potassium channels and lead to a general inhibition of neuronal activity both at the axon terminal and soma (Law *et al.*, 2000). μ-Opioid compounds are generally inhibitory in the thalamus (Brunton & Charpak, 1998) and injection of agonists into the central lateral, parafascicular and mediodorsal thalamic nuclei increase thresholds for both pain and reward (Carr & Bak, 1988). In contrast, MOR agonists administered systemically to humans elevate rCBF in the cerebral cortex rather than reduce it (Adler *et al.*, 1997; Schlaepfer *et al.*, 1998; Wagner *et al.*, 2001; Petrovic *et al.*, 2002; Leppä *et al.*, 2006) and the responses in thalamus have been inconsistent with an increase, decrease or no change in rCBF.

Wagner *et al.* (2001) recognized the conundrum produced by cingulate cortical activation by MOR agonists in the context of a general neuronal inhibition by such compounds. One solution to this conundrum is that MOR mainly inhibit inhibitory interneurons, thus releasing a disinhibition and allowing projection neurons to rise to firing threshold. Since met-enkephalin may be released mainly from interneurons and transmitter autoreceptors usually provide for inhibitory feedback (Sar *et al.*, 1978; Khachaturian *et al.*, 1983), this is a mechanism of action that is consistent with human and experimental animal studies. In addition, adenylyl cyclases 1 and 5 are inhibited by MOR activated Gi/Go proteins and these could mediate the inhibitory actions of MOR that are essential for the actions of morphine (Kim *et al.*, 2006) and mediate long-term potentiation in ACC (Liauw *et al.*, 2005). Of course, it is possible that stimulation of adenylyl cyclases 2, 4 and 7 could account for the stimulatory actions of MOR and these proteins might be differentially expressed by classes of cortical pyramidal and non-pyramidal neurons. This mechanism may be a pertussis toxin-insensitive activation of G-proteins by DAMGO and other inverse MOR agonists (Federman *et al.*, 1992; Chakrabarti *et al.*, 1998; Szücs *et al.*, 2004) and could also account for stimulation of pACC/aMCC by remifentanil and fentanyl.

MOR-driven intracortical cascade circuit

It is not yet possible to provide a complete circuitry that is driven by activation of MOR because many of the experimental rodent studies have not been implemented in monkey. However, some of the key players are available and extrapolation in major systems in the rat may be valid and certainly worth further exploration. Figure 15.5 presents some of the key MOR-regulated systems discussed here and below in terms of descending outputs and from information in the previous chapter on primate pain processing. It focuses on area 24b because ACC has the highest level of MOR binding and this region raises questions about both pain and emotion processing in the context of motor outputs. This excitatory-intracortical cascade model is based on a MOR-triggered disinhibition of multipolar neurons in layer III and a subsequent collateral excitation of motor outputs from layer V. The model is built on an overview photograph of neuron-specific nuclear binding protein (NeuN) immunoreactivity in layers III and Va. Although the NeuN immunoreactivity is not phenotype-specific, it does show all neurons and it is presumed, pending further detailed information about specific classes of neurons, that there are few intracortical neurons in layer Va, where most of them appear to be large and pyramidal in shape. In contrast, layer III has smaller pyramids and there are likely many more intracortical

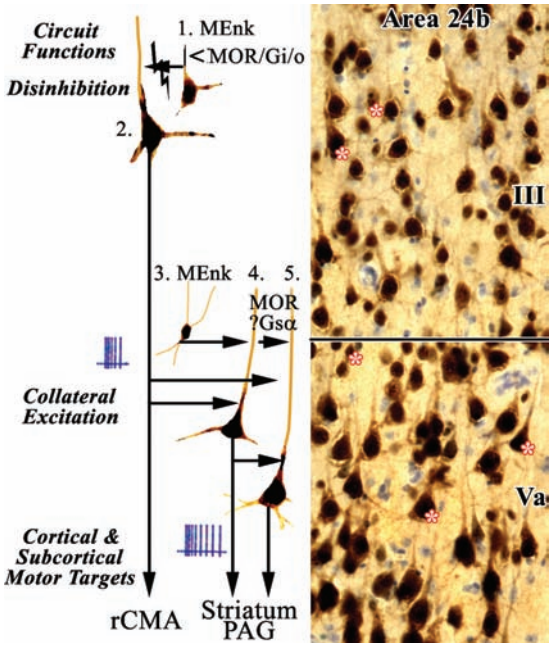

Fig. 15.5 MOR-driven intracortical cascade model for ACC/area 24b. Layers III and Va are of area 24b shown with NeuN on the right and a horizontal line is at their border (MOR are rare in monkey layers I-II/VI and these are not shown). The red-stroked asterisks are next to neurons that were copied for the circuit diagrammed on the left. A cascade of excitation is triggered by disinhibition of MEnk interneurons (1; arrows for projections) via MOR activation (lightning bolt; block of GABA release) and drive cortical projection neurons (2; extracellular spike train in blue). Amplified excitatory output derives from collaterals to motor system projection neurons (4, 5) and a MEnk interneuron (3) may enhance excitatory output via stimulation of Gsα(?) which is not yet established. Although area 24b does not project to the spinal cord, it directly regulates motor system output via the three outputs shown.

neurons. This is supported with matched sections stained for calretinin interneurons which are most dense in layers II and III throughout area 24, although many of these neurons may not themselves be enkephalinergic (not shown in this figure).

Five neurons were copied from the NeuN section on the right for the circuit diagram on the left of Figure 15.5; 1 and 3 are met-enkephalinergic interneurons (MEnk), and 2, 4, and 5 are pyramidal neurons with that in layer III (2) providing mainly corticocortical connections including substantial axon collaterals to layer Va neurons and projections to the rostral cingulate premotor area (rCMA) in the depths of the cingulate sulcus. It is well established that layer III pyramidal neurons emit collaterals in deep layers and these as well as the long efferent connections are shown with arrows, although a specific study of area 24b in human is not available. Finally, layers I–II and VI are not shown because MOR are rare in these layers in primates.

The model proposes that a cascade of excitation is initiated by disinhibition, i.e., inhibition of MEnk interneurons (1) by MOR activation (lightning bolt in figure) which blocks tonic release of GABA and allows cortical projection neurons to achieve threshold for firing (2; extracellular spike train in blue). Excitatory output from layer III pyramids drives collateral discharges in layer Va projection neurons (4, 5), and there is the possibility that MEnk interneurons in layer Va (3) enhance excitatory output via stimulation of Gsα activation of adenylyl cyclases. Although area 24b does not project to the spinal cord, it can regulate motor system output via many projections, three of which are shown in the model. Corticocortical projections are emitted from area 24b to the rCMA as well as subcortical projections to the striatum and PAG. Thus, the cascade of excitation engages layer Va efferent projection neurons that are the primary motor control system driven by a MOR disinhibition in layer III.

In conclusion, MOR regulation in the cingulate gyrus is in a position to modulate many motor system outputs. Since an opiate placebo has been demonstrated in ACC, it is possible that cognitive processes can simulate the actions of opioids probably by intervening in the same circuit described here. Top–down processing, placebo responses, and descending systems are critical elements to the role of cingulate cortex in opioid receptor actions and therapeutics.

Placebo Map

Placebo events are a well-established concomitant of medical interventions and are most profound with opiate drugs and anticipation of pain relief. An early study employed standard post-surgical care and a "special care" group and observed a significant reduction in morphine administration (Egbert *et al.*, 1964). The "special care" patients were informed of their impending pain pre-operatively, and advised on how to manage it with relaxation, exercise, and other methods. They also received special counseling by the anesthetist and consideration of the post-surgical medication options. Although the surgeons operated blind as to which patients were in which group, the "special care" group used only half of the morphine that other patients required.

Not all studies of placebo events, however, observe these responses. A review of 130 clinical trials showed no effect on binary measures of a placebo effect and in small sample size trials and even in large trials, only measures of subjective placebo effects were significant, while those with objective outcome measures were not (Hrobjartsson & Gotzsche, 2001). There was, however, evidence for a significant placebo effect in trials involving pain management. Further experimental

evidence supports the role of placebo effects in pain management (Evans, 1974; Benedetti *et al.*, 1999; Watson *et al.*, 2006).

Parts of the cingulate gyrus have elevated activity in a number of different placebo events, however, they do not always overlap and this suggests multiple mechanisms of the placebo instead of a single placebo. Although the ACC is the most frequently involved, the various placebo responses fit into the four-region neurobiological model and here we present a map of these responses. As new placebos are explored in terms of brain mechanisms, new cingulate localizations should also be identified as each taps into the unique and differential functions of cingulate subregions. The classes of cingulate-mediated placebo events reported thus far include pharmacological (MOR and serotonin uptake), anticipation of emotional relief, and mechanical/acupuncture expectation of pain relief. Here, we identify the areas which are engaged in placebo events, while the role of cingulate cortex in expectancy and anticipation of pain and analgesia is discussed in Chapter 16.

The first assessment of central nervous system activity during a placebo event was that associated with the fast acting MOR ligand remifentanil (Petrovic *et al.*, 2002). This activation was centered quite laterally in the cingulum bundle (their Fig. 1C) and activity in this site was correlated with that in the brainstem; presumably associated with the PAG. Interestingly, this pACC/cingulum bundle site was not the same one activated during noxious stimulation in pMCC in the same study. Although the placebo site did underlie the area of remifentanil activation in the cingulate gyrus, the total area of placebo activation was only about 5% of the total area encompassed by activations generated by remifentanil. Finally, the entire placebo response appeared to be explained by the high responders, i.e., low responders did not contribute meaningfully to the opioid placebo response. Differentiation of the pain activation and placebo sites in the same subjects confirms that the placebo response is not mediated directly by interactions of both in the cingulate cortex but rather by an intermediary site such as the PAG. Additionally, placebo analgesia in patients with irritable bowel syndrome do not appear to be mediated by a naloxone-blockable response (Vase *et al.*, 2005) and suggests mechanisms beyond those associated with opiate drugs.

Another experimental model to study placebo analgesia is the use of a conditioning paradigm in conjunction with an inactive cream (Bingel *et al.*, 2006). This placebo effect was generated by reducing the intensity of the laser stimulator a number of times before the noxious pulse used for imaging. This procedure activated a significant part of the gyral surface in

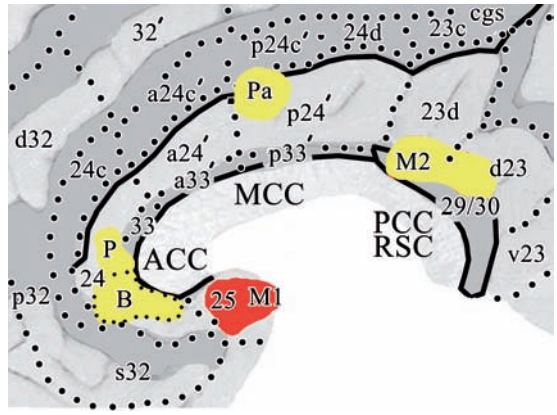

Fig. 15.6 Distribution of placebo responses in the cingulate gyrus co-registered onto a flat map from Chapter 3. Increases over control state shown in yellow and reduction in red: M1, M2 Mayberg *et al.*, (2002); P, Petrovic *et al.* (2005); B, Bingel *et al.* (2006); Pa, Pariente *et al.* (2005).

ACC as shown in Figure 15.6 (B). This site had activity that co-varied with that in the amygdala and a part of the brainstem that likely included the PAG. Thus, not only is ACC involved in the placebo response associated with the expectation of pain relief, this region regulates both opioid and inactive-cream-conditioned analgesia. The ACC, however, is not the only part of the cingulate gyrus that mediates placebo events.

Acupunture placebo

Acupuncture alters CNS activity associated with pain and the expectation of pain relief and a number of studies report localizing an acupuncture placebo site in cingulate cortex. Zhang *et al.* (2003) reported an increase in activity in pMCC during electrical acupoint stimulation; the same region that is active during noxious stimulation. Indeed, Biella *et al.* (2001) showed that true acupuncture (20–25 min of non-noxious needle application) activated the entire pain neuromatrix including pACC and most of these sites including pACC were shared with those activated by a placebo acupuncture condition. They suggested that the analgesic mechanism of action might be due to disruption of processing in this network. In contrast, acupuncture placebo in patients with osteoarthritis, under conditions that do not alleviate pain, activate pMCC. Pariente *et al.* (2005) performed a conjunction analysis of real acupuncture and placebo acupuncture with the Streitberger needle in patients and activated pMCC as shown in Figure 15.6 (Pa). Although the expectation of pain relief in a somatic pain syndrome increased activity in MCC, categorical analyses showed a response rostral to the MCC on the border with pACC. Finally, a midbrain response was generated in the conjunction

and categorical analyses and may provide for a cognitive engagement of descending pain control via the PAG. There are many methodological and analytical differences among acupuncture studies and associated placebo events and understanding of these variables will help to elucidate the multiple roles of cingulate cortex in modulating expected but not delivered (placebo) noxious stimuli.

Emotional placebos

Placebo events are usually acute and evaluated during a single day, however, brain responses over days and weeks have also been evaluated with complex emotional stimuli rather than needles, creams, or drugs during a single study period. A study by Mayberg *et al.* (2002) evaluated the cerebral metabolic rate for glucose (CMRglc) in patients with unipolar depression during a fluoxetine treatment or placebo drug over periods of 1 or 6 weeks. There was no evidence of a placebo effect after 1 week, but after 6 weeks, half of the responders had received the placebo drug. The response with placebo over a 6-week period was associated with an increase in activity in areas 23d and d23 and retrosplenial cortex (RSC) as shown in Figure 15.6 (yellow, M2) and a decrease in activity in area 25 (Fig. 15.5; red, M1). This joint pattern of activity is a striking difference from other studies and could suggest one reason why selective serotonin reuptake inhibitors require weeks to achieve efficacy. The joint placebo response may be critical for many patients and it may require a long time to engage multiple nodes in a network.

In a study of unpleasant pictures with an expected anxiolytic treatment, another placebo response was generated in pACC that was quite close to the opiate placebo (Petrovic *et al.*, 2005; Fig. 15.6, "P"). Since this response is rostral to that identified by a correlation analysis which was located in aMCC, it is possible that there was some divergence in studies involving emotional activations. Fear and anxiety may be generated dorsally in pACC/aMCC, while that associated with a reduction in negative mood due to a reduction in activity (Mayberg *et al.*, 2002) occurs mainly in sACC. Interestingly, the opiate placebo is more closely related to the anxiolytic placebo than that of the selective serotonin reuptake inhibitors. It appears that drug and cognitive drive both influence the cingulate-mediated mechanisms of placebo events.

Do placebos and hypnosis activate MEnk and layer Va pyramidal neurons?

Although neurons that are the targets of cognitive action cannot be observed with functional imaging, placebos and hypnosis may interact in neuronal circuits that are also regulated by opioid peptides. In fact, this must be true to the extent that an opiate placebo site overlaps with high MOR binding. The MOR-driven intracortical cascade model shown in Figure 15.5 does not explain how the circuit is initially triggered, although it does provide that direct administration of exogenous opiate compounds is pivotal to engaging projections to the cortical and subcortical motor systems. It seems that cognitive driving of pain anticipation and pain relief requires an explicit circuit input in this system because the link between the ACC and PAG are so frequently reported in studies of pain control.

There are two means by which cognitive engagement of descending PAG projections might occur. First, cognitive driving of MEnk interneurons may generate the disinhibition which leads to outputs to the PAG according to the model above. Secondly, cognitive attention to pain relief might directly activate the layer Va neurons that project to the PAG and subsequently trigger pain relief. Thus, cognitive attention to pain relief might activate either the MEnk interneuron or cortico-PAG neurons or both together and these links are critical among all mechanisms of pain relief that are mediated by ACC. Needless to say, the mechanism of cognitive control over particular interneurons or projection neurons is not understood. The dual role of cognitive and transmitter system activation in placebo events require that different and explicit circuits will be needed for each class of placebo in each subregion of the cingulate gyrus.

Descending Control Systems

This chapter considers MOR, opiate placebos, and descending control mechanisms because one of the most established methods of intervening in descending control is via the MOR. The principal cingulate output projections are located in layer V and this is one of the major sites of MOR regulation. This does not mean to imply, however, that all descending projections are regulated by these receptors or these are their only functions; only that the positive linkage is likely greater than for the non-MOR expressing output neurons.

Descending control of emotional motor systems including those for vocalization and pain are critical for coordinated and goal-oriented behavioral output and the ACC and MCC are pivotal players in selecting among behavioral outputs and emotional expression via its descending output systems. Descending control via the PAG and PB nuclei has been invoked as a mechanism for generating analgesia in the spinal cord via the diffuse noxious inhibitory system (DNIS), for generating pain control during hypnosedation (Chapter 17), and during various placebo events as discussed above. Another class of descending systems includes projections of ACC to the amygdala for regulating responses to fear (Chapter 9) and the control of visceromotor

outputs (Neafsey *et al.*, 1993; Chapter 10). Finally projections to the hypothalamus provide another means of direct regulation of autonomic output by cingulate cortex; both hormonally and via projections of the hypothalamus to the PB, PAG and spinal cord (Chapter 22). Thus, the cingulate gyrus has a wealth of descending projections to regulate many behavioral outputs and they are evaluated in detail throughout this volume. This chapter considers some major classes of subcortical outputs and links them with the important subregional functions of cingulate cortex.

Since human structural imaging has not yet reached a level of resolution that provides for cellular parcellation of particular subcortical nuclei, photographs are provided here to show the structure and exact locations of key brainstem nuclei in the monkey that receive major anterior cingulate cortical descending projections in Figure 15.7. The sections were immunoreacted for microtubule-associated protein-2 (MAP2) which labels mainly large dendrites and provides good resolution of individual nuclei. Levels through the thalamus, midbrain, and medulla are shown and all abbreviations are provided in the list at the front of this volume. These same sections are used in a number of figures below to assess the projections of cingulate cortex and in other chapters (10, 14, 22). The level through the thalamus is

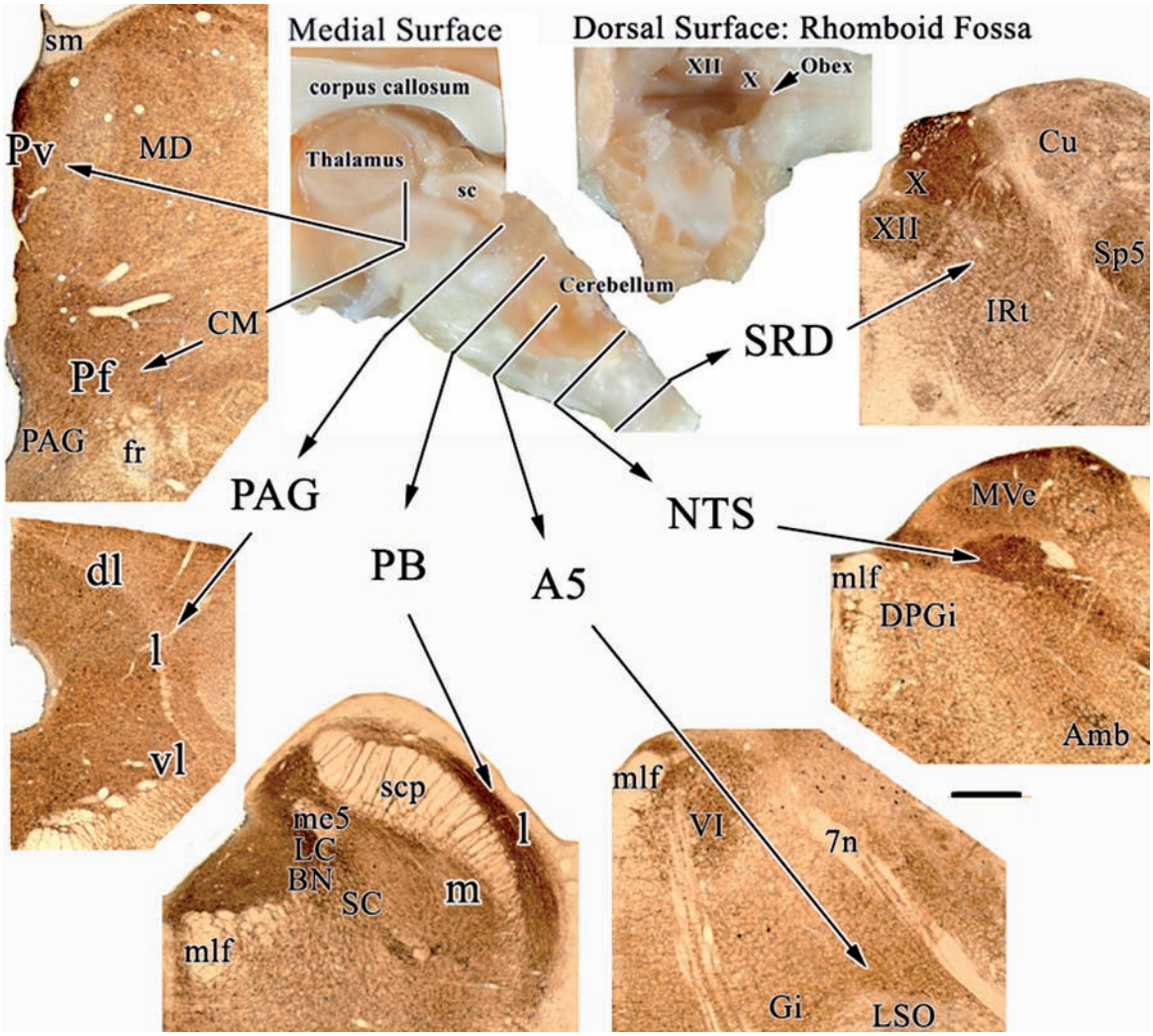

Fig. 15.7 Overview of subcortical nuclei that either project to cingulate cortex or receive cingulate input. Sections reacted for MAP2 at levels on a monkey medial surface. A dorsal view of the rhomboid fossa (cerebellum removed) exposes the 10th and 12th trigones. The levels are of posterior thalamus (Pv and Pf nuclei), the dorsolateral (dl), lateral (l), and ventrolateral (vl) divisions of PAG, the lateral (l) and medial (m) divisions of the PB, A5, nucleus of the tractus solitarius (NTS) and the subnucleus reticularis dorsalis (SRD) also termed the dorsal reticular nucleus. Other structures are labeled with smaller font and abbreviations are in the list at the front of this volume. Calibration, 1 mm.

a caudal one to show the location of the paraventricular (Pv) and parafascicular (Pf) nuclei that are engaged in cardiovascular and nociceptive responsiveness, respectively. The three dorsoventral divisions of the PAG are noted and the high level of immunoreactivity in the vlPAG is associated with heavily MAP2-labeled dendrites. The medial and lateral divisions of the PB nucleus are easily detected and each division has a different linkage with sACC. Further sources of autonomic afferents and cingulate efferents arise in the nucleus of the tractus solitarius (NTS). Finally, although the subnucleus reticularis dorsalis (SRD) has not yet been shown to receive inputs from the cingulate gyrus in primates, it does project to the Pf and is likely a source of nociceptive inputs to this system in the rat as discussed in Chapter 14 and below.

Proximity of "unpleasantness" and "happiness" and relevance to descending control

One of the conundra of co-localization studies of emotion is the very close, if not overlapping, activation sites for "unpleasantness" and "happiness." Lane *et al.* (1997) first showed that area p32 is prominently active while subjects attended to subjective emotional responses. Although area p32 in the rostral pACC (Chapter 3) is most often associated with happiness in studies of simple emotion (Vogt *et al.*, 2003), cognitive amplification of unpleasantness during noxious stimulation enhances activity in area 24 of pACC (Kulkarni *et al.*, 2005). As discussed later, the entire pACC has extensive projections to the PAG and may contribute differentially to the descending regulation of pain and other systems. One solution to the very different functions of pACC is differentiation of function by area as noted, since these functions do not completely overlap. Even with some degree of overlap, the functions may be mutually exclusive for negatively and positively valenced emotions. It is also possible that "unpleasantness or suffering" may be related to shutting off "happiness" either via internal states or by external noxious stimulation. As discussed in Chapter 17, another alternative includes the enhancement of happiness with hypnosis which might shut off nociceptive afferents via the descending noxious inhibitory system. The ultimate value of the four-region model and its subregions derives from its ability to expose these types of mismatches and resolve them with circuitry, cognitive, and pharmacological explanations. For the present purposes, it is important that area p32 is primarily engaged in positively valenced emotional experiences and areas 24, subgenual 32 (s32), and 25 are involved mainly in negatively valenced emotional experiences. This differential coding of ACC outputs regulates emotional expression via projections to other structures such as the PAG. Indeed, these differential projections into the PAG may select the appropriate and pre-wired reflexes associated with the emotional motor output of the PAG.

The cingulate vocalization subregion regulates the PAG

It is well established that the dorsolateral PAG (dlPAG) is involved in vocalization and laryngeal control. Activity in this region is correlated with respiratory output in studies of awake monkeys (reviewed by Larson *et al.*, 1988; Davis *et al.*, 1996). Zhang *et al.* (1994) showed that chemical stimulation of the PAG evoked changes in respiratory, laryngeal, and oral muscles in decerebrate cats. Thus, the PAG is the final common pathway for coordinated vocalizations and may be used by telencephalic structures to coordinate respiratory and laryngeal motor patterns necessary for a wide range of valenced vocalizations including prosody (intonation) in human speech.

Of greatest importance to the present consideration is a study by Jürgens and Pratt (1979) because they early appreciated the pivotal linkage between the "precallosal" part of ACC and the PAG and its role in vocalization. They showed that the cingulate vocalization subregion (CVS) projects to the dlPAG and ablation of this projection blocks conspecific vocalizations elicited with electrical stimulation of the PAG. They also showed that a network of forebrain projections to the PAG, including the CVS and their projections to the nucleus ambiguus, control laryngeal motoneurons. Thus, the pivotal role of the CVS in driving emotionally relevant vocalizations from the PAG has long been appreciated.

The CVS is referred to here as a subregion because it is comprised of parts of at least three cytoarchitectural areas including areas 24c, 24b, and 32 and this is part of the larger ACC region. It is plotted onto a flat map of monkey cortex in Figure 15.8A because this is the species in which most experimental work has been performed. A plot of each ablation site that was effective in altering vocalization is shown in Figure 15.8B along with a summary of three sites that were effective in generating vocalization with electrical stimulation. The effective ablation sites were reported by Aitken (1981) to disrupt conditioned and spontaneous vocal behavior and that by Sutton *et al.* (1974) which affected performance of discriminative calls. Electrical stimulation-evoked vocalization was generated by Jürgens and Ploog (1970), Müller-Preuss *et al.* (1980), and Kaada (1951).

Assessing the site that is pivotal to vocalization must consider that the face division of the rCPMA is located in the rostral and ventral bank of the cingulate sulcus in area 24c. This area projects to the facial nucleus and regulates the muscles of facial expression (Morecraft *et al.*, 1996, 2007). There is also a large part of pACC that

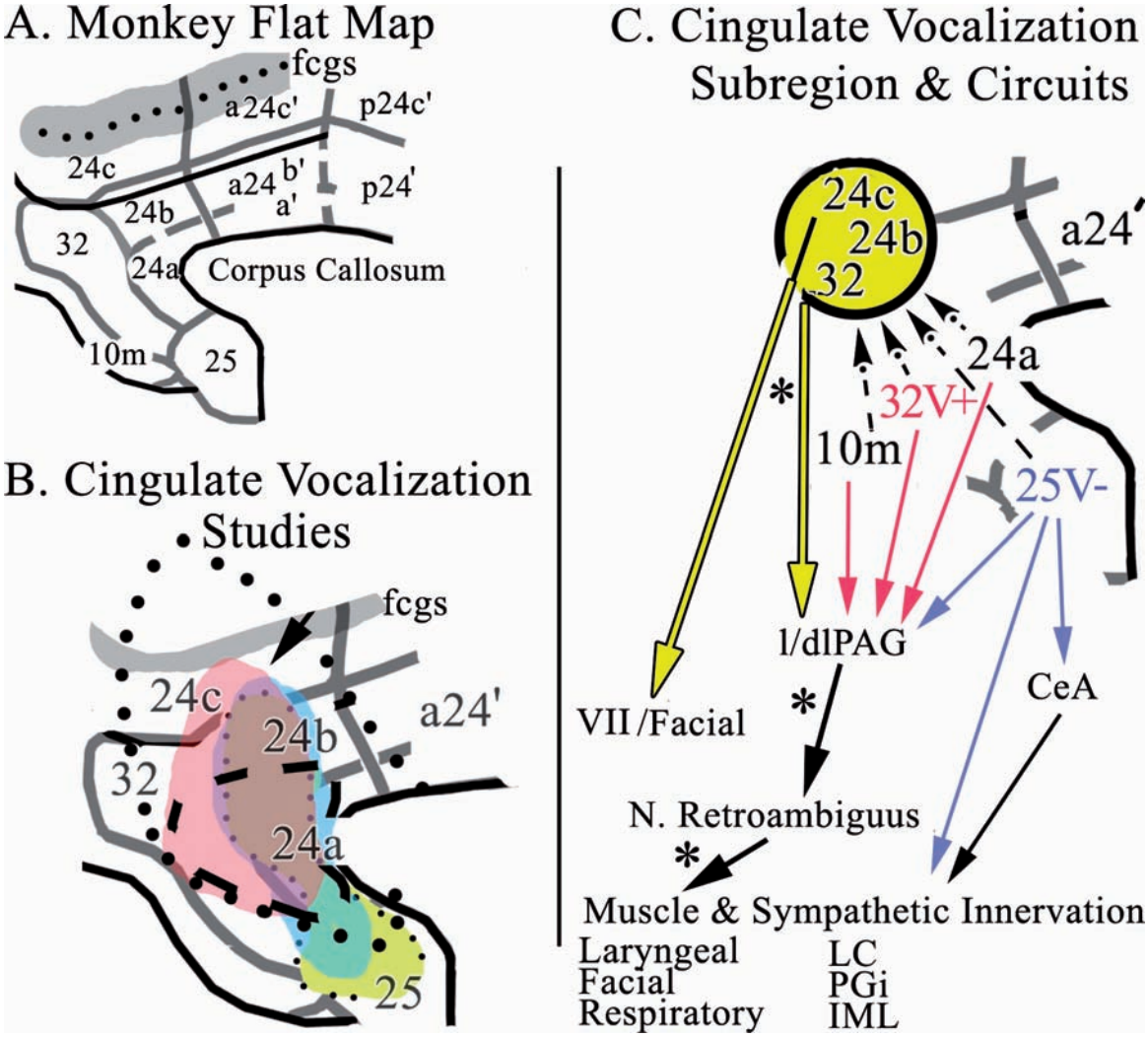

Fig. 15.8 The cingulate vocalization subregion plotted onto a flat map of monkey cortex (A). (B) Plots of ablation impaired [dashed line, Aitken (1981) conditioned and spontaneous vocal behavior; dotted lines, Sutton *et al.* (1974) performance of discriminative calls] and electrical-stimulation-evoked vocalizations (yellow outlined with small dots, Jürgens & Ploog, 1970; blue, Müller-Preuss *et al.*, 1980; red, Kaada, 1951). (C) Pivotal to defining the CVS (yellow) are projections (asterisks) from area 24c to the facial motor nucleus and of areas 24c, 24b, and 32 through the l/dlPAG to the retroambiguus nucleus and motor neuron pools that regulate laryngeal, facial and respiratory musculature. Multiple medial surface inputs to the CVS are shown with black dashed lines that support positively or negatively valenced outputs. Secondary projections from area 25 and the CeA support autonomic output via projections to the LC, PGi, and IML. The 32V+ represents the positive emotional nature of vocalizations from area 32 and 25V– emphasizes the negative nature of vocalizations from area 25.

contains neurons with activity that is synchronized to vocalization, jaw opening, and other oromotor activites (West & Larson, 1995). Of course, the lesions and electrical stimulation sites likely overstate the exact subregion distribution and we localize the CVS to the dorsal part of pACC as shown in the Figure 15.8C (yellow). Detailed arguments for the role of this region in emotional vocalizations and demonstration of its auditory input from temporal auditory areas TS1, Pro, TS2, and TS3 have been reported (Vogt & Barbas, 1988) and Chapter 6 has a review of these connections.

The PAG is a final common pathway by which forebrain structures drive vocalization for intonation in speech and emotional communications. These motor outputs are associated with nocifensive responses as during noxious stimulation including facial expressions and vocalization of "ouch!" and sexual expressions including the cooing and facial expressions of pure joy. As summarized in Figure 15.8C, the l/dlPAG projects to the retroambiguus nucleus and motor neuron pools that regulate laryngeal, facial, and respiratory musculature. Secondary projections from area 25 and the central

nucleus of the amygdala (CeA) support autonomic output via projections to the LC, paragigantocellular (PGi), and intermediolateral spinal cord (IML) nuclei. The figure also emphasizes that emotional content is pivotal to PAG inputs that arise from the CVS. Thus, the positive emotional nature of vocalizations generated from area p32 and the negative emotional nature of vocalizations generated from area 25 are shown based on discussions earlier about the distribution of emotional activity during happy and sad events. Vocalizations mediated by ACC all have valence, contextual, and behavioral relevance and likely provide an important substrate for prosody during speech. Pivotal links between conspecific vocalizations including infant/maternal interactions and those emitted during sexual behavior have been discussed in great detail in relation to the anterior cingulate gyrus by MacLean (1990).

Efferent Systems Drive Descending Noxious Inhibition: PB and PAG

Cingulate cortex responses to noxious stimulation are not limited to driving skeletomotor outputs like those for vocalization because this region has profound projections to the Descending Noxious Inhibitory System (DNIS) and many autonomic nuclei including the CeA that regulate autonomic output. Additionally, there is evidence for cingulate projections into the Descending Noxious Facilatory System (DNFS) that is pronociceptive as discussed below. Thus, "top–down" regulation of nociceptive responses may provide for either override inhibition or enhancement of pain processing that is generated by cognitive assessment of expected behavioral outcomes. Human imaging studies often co-activate cingulate cortex and the PAG in a mechanism for reducing central pain processing as discussed, for example, for the acupuncture placebo above and hypnosis in Chapter 17. The following consideration reviews the specific structures involved in these descending systems for regulating nociceptive and autonomic circuits.

Parabrachial Nucleus

The PB nucleus is important because it provides visceral nociceptive information to the parafascicular nucleus which in turn projects to ACC (Chapters 10 and 14) and it projects to the DNIS. The PB receives input from areas 25 and 32 of ACC (Yasui *et al.*, 1985; Chiba *et al.*, 2001) and it projects to the spinal cord and mediates noxious inhibitory responses. Girardot *et al.* (1987) reported that electrical stimulation of PB inhibited noxious and innocuous activity in thoracic, spinothalamic tract neurons including those that project to medial thalamus and this provides a route via which cingulate cortex can mediate descending inhibitory functions. Further, excitation of the PB with carbachol in conscious guinea pigs doubles the duration of restraint-induced tonic immobility and reduces motor defense and vocalization normally evoked by noxious electrical stimulation of the skin (Menescal-de-Oliveira & Hoffman, 1993). Thus, the PB is a pivotal intermediary to the nociceptive properties of cingulate cortex by providing a source of viscerosensory input to Pf and it participates in the DNIS. Since the PB receives input from area 25, it is a cingulate intermediary that may provide for the conscious regulation of the DNIS as summarized in Figure 15.9.

Cingulate projections to the PAG

The PAG has a key role in the DNIS and layer V neurons of the anterior cingulate gyrus project to the PAG (Müller-Preuss & Jürgens, 1976; Hardy & Leichnetz, 1981; Mantyh, 1982). These projections are from layer Va of multiple areas of the ACC and aMCC including areas 25, 32, 24, and a24′. A summary of the important findings of An *et al.* (1998) is provided in Figure 15.10, where the density of these projections are coded with the level of shading in layer V on the left and by the size of the arrows on the right. The most dense projections (i.e., number of retrogradely labeled neurons) to the dlPAG arise from areas 32 and 25. Moderate projection densities are from area 24b/c and the lowest density was to vlPAG from a24b′. Obviously the projection

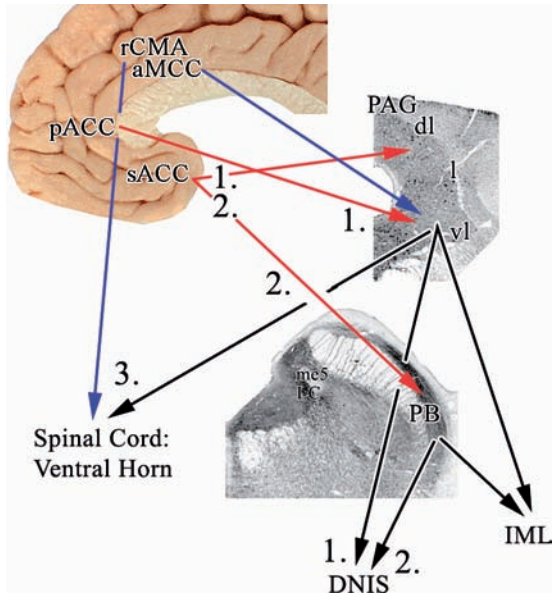

Fig. 15.9 Two descending cingulate projections regulate the DNIS: (1) Both ACC and the aMCC project to the PAG for coordinating autonomic and skeletomotor output from the spinal cord and (2) sACC projects to the PB for coordinating autonomic outflow via the intermediolateral spinal cord (IML). (3) a MCC and PAG project to the spinal cord as part of skeletomotor control system.

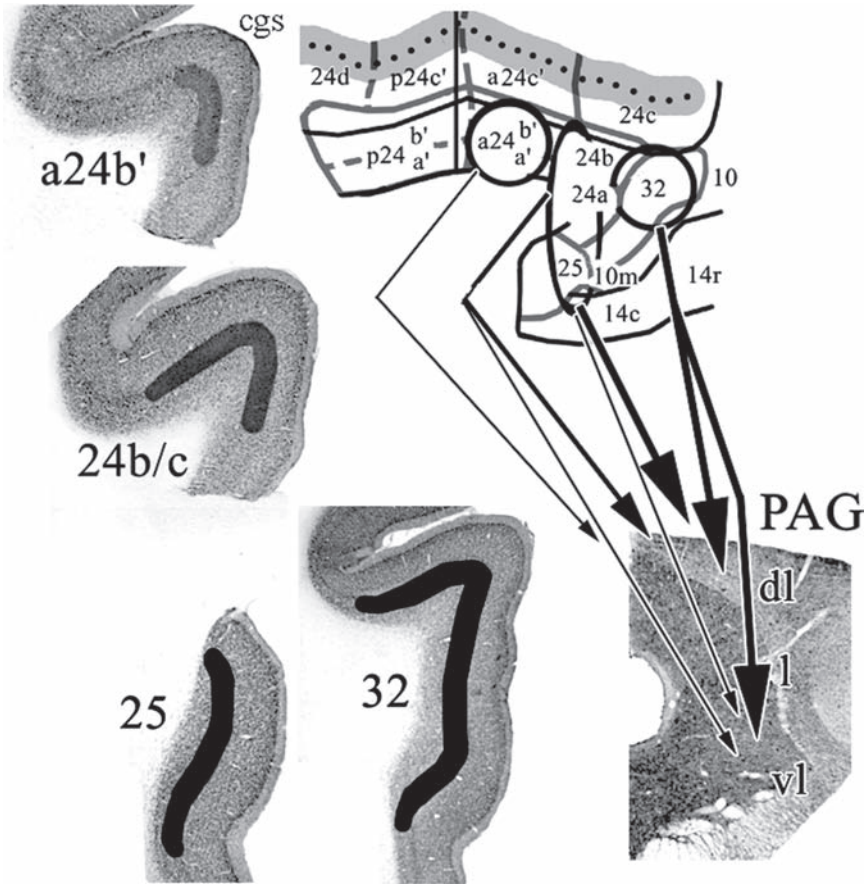

Fig. 15.10 Summary of An *et al.* (1998) on a flat map of monkey cingulate gyrus (Vogt *et al.*, 2005). The relative contributions of progressively more caudal parts of cingulate cortex to the PAG is heaviest from area 32, moderate input arises from area 24 and least from area a24a′b′. The density of the projection is shown with the intensity of gray/black in layer Va on left and arrow sizes on the right.

is greatest from the most rostral parts of the cingulate gyrus and they decrease in density at caudal levels. This disproportionately large input from area 32 may be critical to the mechanisms of hypnosedation based on induction methods using pleasant life experiences as discussed in Chapter 17. It is also interesting in terms of the pattern of MOR binding in the primate with greatest densities in ACC, less in MCC, and least in PCC.

As noted earlier, one of the reasons for including opioid receptor binding in the same chapter as descending control systems is that the former regulates the latter. Indeed, co-localization of MOR binding in layer V that is around large pyramidal neurons that project to the PAG (Figs. 15.3 and 15.10) is the pivotal linkage for the actions of opiate compounds on this system; although direct synaptic connections have yet to be demonstrated. Injections of naloxone into the ventrolateral PAG (vlPAG) impair fear extinction in a dose-dependent manner (McNally *et al.*, 2004); conditioning processes that are likely dependent on sACC and the amygdala. Also, this part of the PAG regulates freezing and conditioned fear responses (De Oca *et al.*, 1998). For these and many other reasons, the PAG has been viewed as a pivotal player in coordinating emotional motor system outflow (Holstege *et al.*, 1996). Thus, key decisions on behavioral output are made in ACC and aMCC in relationship to internal needs and anticipated outcomes and these regulate the pre-wired output of the brainstem emotional motor system to produce context relevant output. The MOR regulates emotional motor systems in the anterior cingulate gyrus, PAG, amygdala, and PB for coordination of many emotion-relevant motor outputs.

Cingulate cortex may inhibit the PAG to block the DNIS

Although human studies show correlative cingulate-PAG activation during states of reduced pain perception, an interesting study by Toda (1992) reports that electrical stimulation of ACC inhibited PAG neurons that responded to both noxious and innocuous somatic stimulation. He proposed that the inhibitory mechanism in the PAG was mediated by GABAergic neurons because bathing them in the GABA receptor antagonist bicuculline blocked the inhibitory response. Although similar findings are not available in primates, it must be considered particularly in chronic pain and stress syndromes, that the PAG connection may not always be

associated with an excitatory/analgesic response, but could also inhibit PAG output and result in a loss of this descending inhibitory response via disinhibiiton of PAG either through the raphe nuclei or other projections to the spinal cord.

Cingulate Cortex Influences Pronociceptive Subnucleus Retricularis Dorsalis: Pain Facilitation

It has been shown in rodents that electrical stimulation of MCC (area 24′) reduces responses of dorsal horn neurons to mechanical stimulation (Senapati *et al*., 2005) as would be expected for the DNIS. There are other reports, however, that both ACC and MCC mediate a facilatory pain pathway via the dorsal reticular nucleus (Zhang *et al*., 2005); also known as subnucleus reticularis dorsalis (SRD). In this latter study electrical stimulation of area 24b enhanced C-fiber-evoked field potentials in the spinal cord and electrolytic lesions of SRD blocked the facilitation. Calejesan *et al.* (2000) also showed that electrical or chemical stimulation of area 24′ facilitated the tail-flick reflex and observed no antinociceptive effects from similar stimulation. The extent to which the DNFS and DNIS are segregated in the rodent cingulate cortex has not yet been established.

The SRD is a site of heterotopic nociceptive convergence in rat (Villanueva *et al*., 1988) and monkey (Villanueva *et al*., 1990) and it may be pronociceptive. Lima and Almeida (2002) provide a thorough overview of the circuitry and functions of the dorsal reticular nucleus. It has been proposed that projections from the SRD to the thalamic reuniens and parafascicular nuclei (Villanueva *et al*., 1998) may contribute to nociceptive activation of ACC (Vogt, 2005).

Hurley *et al*. (1991) and Almeida *et al*. (2002) show a projection from area 25 to SRD in the rat and weak projections from areas 24 or 24′, although Desbois *et al*. (1999) were unable to identify cingulate projections to SRD even from area 25. It is possible there are two descending cingulate systems that modulate pain processing via the reticular nucleus; however, the differential role of each cingulate region has not been

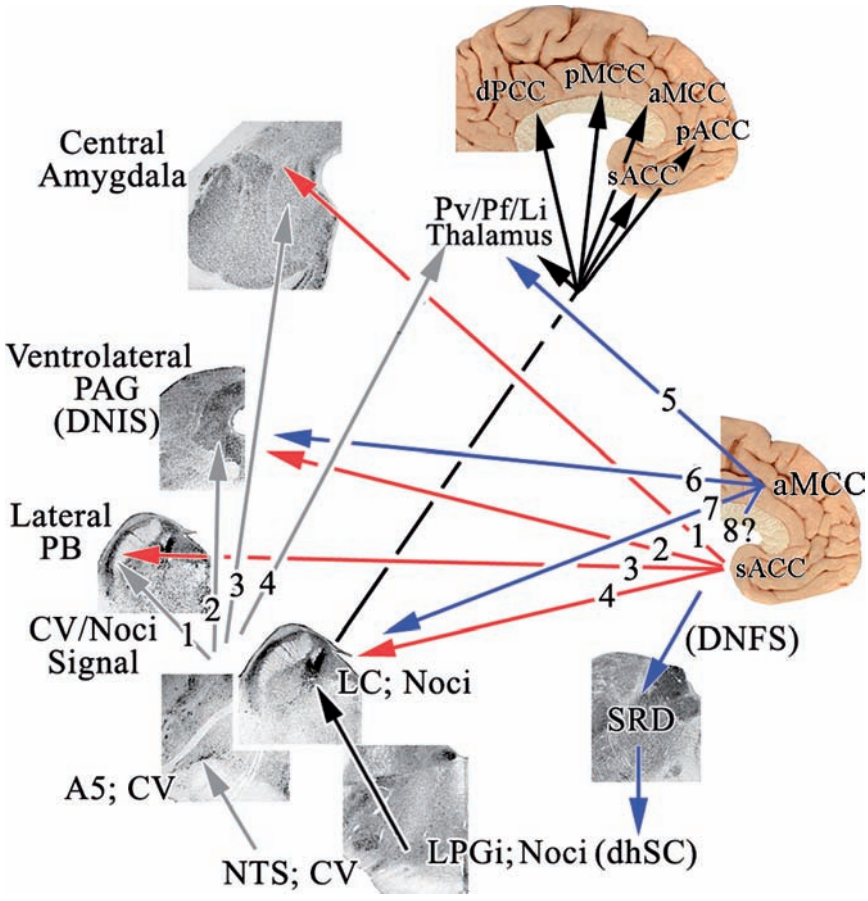

Fig. 15.11 Summary of descending cingulate cortex influences in NEergic regulated nuclei. Ascending nociceptive projections are numbered 1–4 (gray arrows) and descending cingulate projections are grouped into those arising from the sACC (1–4) and aMCC (5–7). This illustration emphasizes outputs from each cingulate region that provides for functionally unique regulation of NEergic systems and the parallel projections to the DNIS and DNFS. Pathway 8 from aMCC is shown with a "?" because it is not known if this subregion in monkey projects to the SRD, although it has been shown in rat.

established and future studies will need more care as to which regions and areas are so engaged, i.e., sACC, pACC, MCC. In addition, it is not known if such projections exist in primates. Although the role of the DNFS in cingulate-mediated pain processing is not known, its presence could have a profound effect on pronociceptive functions in chronic pain and stress disorders. The relevance of this system in the "nocigenic" model of functional pain disorders is discussed in Chapter 22. In order to demonstrate its position in descending systems, Figure 15.11 shows projection #8 from the aMCC with a question mark for primates through the SRD and subsequent projections, either direct or indirect, to the spinal cord.

Summary of descending systems that regulate autonomic and nociceptive processing

Chapter 22 reviews the subcortical nuclei that both receive the highest density of NEergic inputs and significant inputs from cingulate cortex. The view of that chapter emphasizes the overlap of locus coeruleus (LC/allostatic), nociceptive, and autonomic systems and provides critical points of vulnerability to chronic pain and stress disorders. Since cingulate regulation of NEergic, pain, and autonomic systems is a pivotal part of cingulate descending control, we consider the organization of these projections in Figure 15.11.

Figure 15.11 is built around ascending nociceptive and cardiovascular (heart rate) projections mediated by NEergic projections reviewed in Chapter 22. Chapter 14 presents nociceptive projections via the MITN (e.g., Fig. 14.6) and the four-numbered pathways in Figure 15.11 (gray tone) summarize this organization: (1) PB; (2) vlPAG; (3) CeA; (4) MITN including the Pv, Pf, and Li nuclei. The diffuse cingulate projections of LC are also shown in the context of noxious stimulation, although this is not the only source of sensory activation of the LC. The figure also summarizes the key outputs from sACC (red arrows; 1–4) and aMCC (blue arrows; 5–8) to autonomic and pain processing structures including the DNFS (8?). Pathways "2" and "6" from cingulate cortex to the PAG have been discussed and are shown to emphasize a parallel system to the DNFS. It is well established that electrical stimulation of the PAG evokes a diffuse analgesia that is associated with inhibition of dorsal horn sensory neurons throughout the spinal cord and these phenomena have been reviewed for the PAG by Carrive and Morgan (2004). Thus, there appear to be both descending inhibitory and descending facilatory efferents that originate in cingulate cortex. The latter projection may be particularly important in terms of cognitive selection processes including anticipation of pain as discussed in Chapters 12 and 16 and chronic pain and stress syndromes discussed in Chapter 22.

Cingulate Projections to the Amygdala

As a limbic structure, the amygdala maintains valenced codes of sensory stimuli including those that evoke fear and pain and generates reflexive responses thereto. The role of the amygdala in fear conditioning and its modulation by cingulate cortex is provided in Chapter 9 and its circuitry in Chapter 6. Furthermore, a pivotal finding in post-traumatic stress disorder is that reductions in ACC activation are associated with dyscontrol of the amygdala which generates amplified responses to fearful stimuli such as those which generated the stress disorder in combat as discussed in Chapter 21. Although many thorough reviews are available on its circuitry and functions of the amygdala (e.g., Aggleton, 2000), the purpose of this section is to place the amygdala in the context of emotion and pain processing as they link to cingulate cortex.

Common targets of the ACC and amygdala in the vlPAG contribute to fear conditioning that is mediated by opioid receptors (i.e., naloxone-blocked impairments; McNally *et al.*, 2004). The amygdala and cingulate cortex are jointly engaged in nociceptive processing, are heavily interconnected (Amaral & Price, 1984; Vogt & Pandya, 1987), and both project to similar parts of the vlPAG (Carrive & Morgan, 2004) and PB nuclei. Nevertheless, a paradox linking these structures to pain processing emerges from a study of forebrain activity during noxious thermal stimulation. Derbyshire *et al.* (1997) reported that, whereas ACC has increased rCBF, the amygdala has reduced blood flow. The dissociation of cingulate connections also suggests these regions play a different role in pain processing and, although they are likely co-driven via a common input from the MITN, there may be no interchange of nociceptive information between these structures.

One approach to the amygdala in pain processing is to consider it from the perspective of two sets of cingulate cortex connections into the central, dorsal and medial nuclei of the amygdala. One set of connections arises from area 25 and terminates directly in the medial part of the central nucleus (CeM; Yasui *et al.*, 1985), while a second set arises from areas 24 and a 24′ and terminates mainly in the basolateral and accessory basal nuclei (Vogt & Pandya, 1987). Figure 15.12 shows these connections with subgenual area 25 and aMCC with blue arrows.

The three major sources of nociceptive inputs to the amygdala are shown in Figure 15.12 with red arrows. The amygdala contains nociceptive neurons particularly in CeM (Bernard *et al.*, 1992) and nociceptive afferents arrive from three sources and each has connections with cingulate cortex. The first stream is from the PB (Pritchard *et al.*, 2000) that includes both cutaneous and visceral receptive fields (Bernard & Besson, 1990;

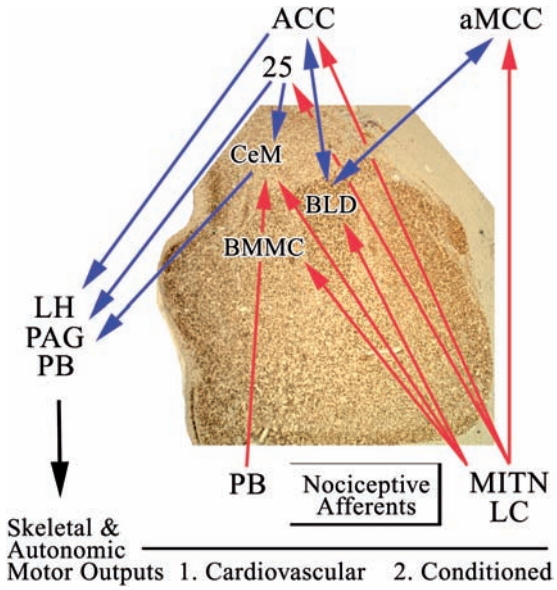

Fig. 15.12 Amygdalar nociceptive afferents and interactions with cingulate cortex. Nociceptive information is derived from the PB, MITN, and LC (red arrows). The sACC area 25 interacts mainly with the CeA, while the aMCC engages mainly with the BLD and AB (not shown). Differentiation of sACC, PB inputs to CeM are paramount to distinguishing projections and information flow through the amygdala and thence to autonomic and skeletal motor centers. Although there appears to be no interchange of nociceptive information between cingulate cortex and the amygdala, these connections serve an important role in fear conditioning. LH, lateral hypothalamus, BLD, basolateral dorsal nucleus, BMMC, basomedial magnocellular nucleus.

Bernard *et al.*, 1994). The CeA regulates autonomic reactions including tachycardia and elevated blood pressure and hypoanalgesia (Davis, 2000). Thus, the first stream is viewed as a direct throughput to autonomic and somatic motor systems (Fig. 15.12) and this pathway modulates conditioning to generate simple significance codes or valences for sensory events possibly in conjunction with projections from sACC.

The second stream of nociceptive information arises from two inputs that also project directly to cingulate cortex and have a wider distribution in the amygdala; thus, these inputs mainly coordinate cingulate and amygdalar outputs without requiring that they interchange nociceptive information between themselves. The two components of this input arise from the MITN (Su & Bentivoglio, 1990) and locus coeruleus (Chapter 22); the latter of which is nociceptive and likely provides for nociceptive driving throughout the forebrain (Hirata & Aston-Jones, 1994). Although the basolateral nucleus (BLA) also receives input from the MITN, it does not directly drive autonomic output and is involved in fear conditioning and discriminative avoidance learning (Maren *et al.*, 1991; Poremba & Gabriel, 2003) as is ACC (Gabriel, 1993). Since fear activity may be mediated via the BLA, while the CeA does not (Koo *et al.*, 2004), this may corroborate a synergistic interaction between aMCC which is involved in fear (Vogt *et al.*, 2003) and the BLA even in humans. It is interesting that only the anterior part of MCC receives amygdala afferents and not pMCC (Vogt & Pandya, 1987).

Figure 15.12 shows the two parallel nociceptive inputs to the amygdala, interactions with the cingulate gyrus, and outputs via two pathways; one involved in cardiovascular control and one that is plastic based on involvement of dorsal cingulate areas in conditioning. Thus, the amygdala is critically linked with ACC and MCC and likely has coordinated roles in generation of fear, however, each contribute differentially to the cardiovascular and conditioned outputs to the brainstem. Finally, conditioning in the amygdala can drive autonomic outputs from the CeA via intrinsic connections (Aggleton, 1985).

In conclusion, the amygdala and ACC/MCC are simultaneously activated by nociceptive inputs via the MITN but probably do not interchange nociceptive information based on responses in human and their reciprocal connections. In contrast, they both engage in conditioning the negative valence of fear-generating stimuli. Disruption of the interactions between these structures may account for enhanced fear and elevated amygdalar activity in the face of reduced ACC activation in post-traumatic stress disorder (Chapter 21).

Tripartite Cingulate Descending Behavioral Control Systems

Fundamental to the four-region neurobiological model is the view of functional specializations for each region. Thus, ACC directly regulates autonomic output and stores valenced, emotional memories, while MCC controls skeletomotor output and is the seat of cognitive response selections as discussed in Chapter 1. In defining the circuit substrates and their functional properties, it is necessary to understand overlaps between the pACC and aMCC subregions from two perspectives. First, as conceptualized for the CVS, there are systems that drive skeletomotor systems with the primary goal of expressing internal states, i.e., emotion. Emotion is clearly expressed by vocalization and facial expressions; indeed, facial expressions are paramount to testing for emotional responses such as fear as discussed in Chapter 9. Both of these functions are driven by inputs of the "face" division of the rostral CPMA that lies in area 24c of pACC. Secondly, some behavioral outputs require avoidance of predicted outcomes such as nocifensive responses. Although these require skeletomotor driving from the "arm" and "leg" divisions of the rostral CPMA in area a24c′, they also require an emotional signal. Thus, the amygdala projects to the aMCC and a fear

response is prominent in aMCC that is unique to the entire cingulate gyrus. This is not a fear response in the sense of affect *per se*, but rather a fear signal that drives skeletomotor output/avoidance behaviors.

To accommodate this important overlap in motor functions and descending control by the pACC and aMCC, the descending circuitry can be conceptualized in terms of a tripartite system. (1) autonomic-specific outputs from sACC, (2) emotional/skeletomotor outputs from pACC including the "face" division of the rCPMA, and (3) skeletomotor/emotional outputs from dorsal aMCC. Figure 15.13 summarizes this circuitry and is based on the detailed discussions of the underlying circuitries in many chapters in this volume. We will not review again the detailed literature for each specific connection but consider only the major sources that have not been generally detailed throughout this volume. Once again, there is an emphasis on summarizing projections in the context of the tripartite system. Also, notice that although the dPCC plays a pivotal role in skeletomotor functions through its interactions with the caudal CPMA, the four-region model predicts an orientation in space and cognitive guiding of behavior through intentions including inputs to the rCPMA as discussed in detail in Chapter 13 and, as such, is not part of the tripartite executive motor systems evaluated here.

When considering Figure 15.13, it is useful to search for projections that differentiate each region. Projections to the hypothalamus, prominently excluding the paraventicular nucleus, originate from each region and appear to terminate in the anterior and lateral areas as discussed in Chapter 10. The locus coeruleus and PAG also have prominent inputs from all three subregions. In contrast and as discussed in each following paragraph, descending projections that differentiate among these three subregions include those to the striatum, amygdala, red and pontine nuclei, raphe nuclei and spinal cord. The most distant projections in primates are not fully understood yet, and those of sACC to the NTS and aMCC to SRD are a matter of ongoing investigations. Thus, both are shown with question marks at this time.

The striatum receives fundamentally different inputs from the sACC when compared to the other subregions. According to the concept of an "open loop" system discussed in Chapter 28, this projection terminates uniquely in the striosomes via the striosomal pathway and from there to dopaminergic reward systems. In contrast, projections via the accumbens (pACC) and subthalamic (aMCC) pathways are part of the "closed loop" system which project in turn to the accumbens and subthalamic nuclei, respectively.

Inputs to the amygdala are briefly discussed above and the critical aspect of cingulate projections thereto

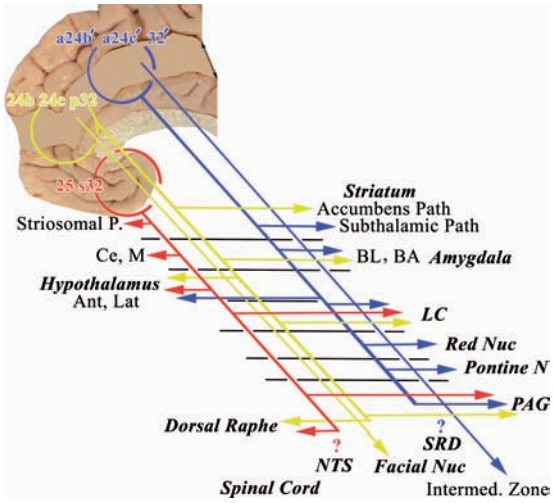

Fig. 15.13 Summary of efferent systems from sACC (red), pACC (yellow) and aMCC (blue) with subregions plotted onto a partial flat map of medial cortex with the cingulate sulcal cortex flattened in the orientation shown with the two-headed arrow. Each major structure is in italics and horizontal, black lines separate some levels of the nervous system. Key differences in outputs resolve around the striatum, amygdala, red and facial nuclei and the spinal cord. Projections to the facial nuclei and spinal cord arise mainly in layer Vb and are shown separately from other projections of layer Va. The NTS projection of sACC is well described in rat but not monkey (?) as is the case for SRD (?).

is the differentiation of the sACC projections to the central and medial nuclei, while the pACC and aMCC project mainly to the basal accessory (dorsal part) and basolateral nuclei. These projections emphasize the direct autonomic regulation of sACC and the role in conditioning and interactions with lateral neocortical afferents by the pACC and aMCC.

Van Hoesen *et al.* (1993) showed a heavy projection of area a24c′ to the red nucleus and proposed this is associated with its role in forelimb movement. Although there is a small projection to the red nucleus from the caudal CPMA as well, no other part of the cingulate gyrus projects to the red nucleus and this is a unique projection among structures of the limbic system.

All cortical regions that regulate skeletomotor functions, as well as most non-primary somatosensory parts of the parietal lobe, in the monkey project to the pontine nuclei (Glickstein *et al.*, 1985). This includes the CPMAs and some gyral cortex of the MCC and dPCC. It is a striking fact that ACC does not project to the pontine nuclei and this difference is shown in Figure 15.13. Thus, ACC may not be engaged in functions mediated by the pontine nuclei and their projections to the cerebellum.

The dorsal and median raphe nuclei receive direct inputs from areas 25 and 32 (Freedman *et al.*, 2000; Chiba *et al.*, 2001) and ACC in general. It is possible that

these projections coordinate some aspects of visceral function and are involved in mood regulation, but there is no experimental work available on the function of this descending system.

The facial motor nucleus receives direct inputs from the face division of the rostral CPMA (Morecraft *et al*., 1996, 2007). In view of its placement in the emotion/ACC region and its overlap with the CVS discussed above and in the previous chapter, it is proposed to play an important role in the facial expression of emotion such as anxiety associated with noxious stimulation. Indeed, this is one of the primary means by which primates share their internal states with conspecifics.

Finally, the motor outputs of each subregion are unique and emphasized at the bottom of Figure 15.13. First, the sACC projects to the NTS in the rat as reviewed in Chapter 10 and play a role in regulating autonomic function along with the central nucleus of the amygdala. Although some investigators have not seen this projection in monkeys (Freedman *et al*., 2000), others have in pilot studies (Chiba *et al*., 2001). Second, as already noted; the pACC projects to the facial motor nucleus and regulates the muscles of facial expression. Finally, spinal efferents from the rostral CPMA in the aMCC project to the intermediate laminae (Dum & Strick, 1996; Morecraft *et al*., 1997). In addition, the caudal CPMA in pMCC and dPCC terminates in a medial part of the spinal cord and suggests differential regulation of motor output by the two motor areas as suggested by Morecraft *et al*. (1997). The CPMA might even project into the intermediolateral nucleus in the thoracic spinal cord to regulate autonomic output and others of these projections are in a position to influence sensory processing in addition to motor interneuronal pools.

In conclusion, the detailed projections from each subregion of the anterior cingulate gyrus have been detailed throughout numerous experimental studies. The specific role of each projection in sensory and motor outflow is not yet fully understood and the challenge for human imaging and experimental animal research is to winkle out the role of each in behavioral expressions of the emotional motor systems. Coordinated regulation of these systems throughout the entire nervous system is possible with psychotropic drugs as well as those that regulate pain. The MOR, for example, is highly expressed in ACC, aMCC, amygdala, hypothalamus, MITN, striatum, nucleus accumbens, locus coeruleus, PAG, NTS, and spinal cord. Pharmaceutical intervention on a system wide basis is possible as is a more system specific intervention such as hypnosedation via projections of the pACC to the PAG. Although human imaging does not yet have the resolution to identify the contributions of each structure and circuit to particular functions, the challenge is to increase resolution and to use the experimental animal observations to guide both study design and data interpretation so that more realistic information can be derived.

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