List of appendices

Appendix 10

Ascending modulatory systems

A10.1 Introduction

We have noted four ascending modulatory inputs to the defence system: serotonergic, noradrenergic, cholinergic, and dopaminergic. Each of these arises in small subcortical nuclei, but then innervates not only the periaqueductal grey, hypothalamus, amygdala, and septo-hippocampal system but also many other subcortical areas and much of the neocortex. This diffuse and widespread termination of multiple collaterals originating from such modest sources suggests that each of these inputs provides a general signal which modulates the target structures rather than detailed information. All the currently known anxiolytic drugs have been shown to interact with the serotonergic and noradrenergic systems. We argue below that the anxiolytics achieve common functional effects on these systems, though often through different primary actions. We will therefore deal with these two systems first and most extensively. The cholinergic system has not been specifically implicated in anxiety, but it is important for an understanding of the more mnemonic aspects of our theory of hippocampal function. Also, anticholinergic drugs are potentially linked to anxiolytics in that they too possess the capacity, albeit qualitatively different, to interfere with theta activity. We will therefore discuss the cholinergic system last. Dopaminergic systems appear to be mainly involved in reward, antidopaminergic drugs are not anxiolytic, and the evidence for a functional role of dopamine in the hippocampus is minimal, so we will not discuss these further.

We will conclude, below, for each of the three aminergic systems that their primary effect on the septo-hippocampal system is to increase its signal-to-noise ratio. Why, then, is more than one system required? There are three answers to this. First, each system has rather different eliciting conditions and operating characteristics. They therefore provide a set of parallel ‘rules of thumb’ which, operating together, allow appropriate responding over a wider range of circumstances than could any one such system operating alone. Second, while they have similar effects on the septo-hippocampal system, they can have diametrically opposed effects on different parts of the more global defence network. As with hormones, then, these systems carry fairly general signals which are used by their different targets in different ways. These targets, moreover, extend beyond the septo-hippocampal system and are much wider even than the defence system taken as a whole.

We consider two different questions in relation to these ascending systems.

First, what can they tell us about the functioning of defence systems? They contribute input to the various areas we have already discussed, and the nature of that input should provide some clues to function. Further, removal of their input should produce at least partial dysfunction of the target areas affected. The tests on which this

removal does and does not match the amygdaloid or septo-hippocampal syndromes should be illuminating. Since these systems target many other areas of the brain as well as the defence system, our assessment of their role in defence will be usefully constrained by the specific, presumed single, function common to these modulatory systems in their effects on both defensive and non-defensive targets.

Second, to what extent could these systems contribute to the effects of anxiolytic drugs? We have proposed that the key area through which many of the effects of the anxiolytic drugs are ultimately mediated is the septo-hippocampal system, and particular its rhythmic theta activity. But we do not suggest that the drugs act only, or necessarily, on the septo-hippocampal system itself to produce these effects. An action on one or more inputs to this system could be sufficient to account for the observed neurophysiological and behavioural effects of the drugs. We have already argued that at least some of the direct effects of the anxiolytic drugs on the amygdala change the information sent from the amygdala to the hippocampus, hence changing hippocampal function in this way. Like the amygdala and the septo-hippocampal system, serotonergic and noradrenergic systems have each been suggested as the primary substrate of anxiety or the primary target of anxiolytic drugs.

In the case of the *classical* anxiolytic drugs, involvement of one or more of the ascending systems is likely, since GABA is widely distributed in the brain. All classical anxiolytics act as direct GABA agonists and/or as potentiators of GABA, and so would increase inhibition in their target structures. This mode of action should, in many cases, produce effects equivalent to those of a functional lesion of afferents to the septo-hippocampal system, amygdala, etc. We will consider the possible role of projection systems using GABA later. But, with all of the other systems, we have to ask how far their function is modulated by inhibitory GABAergic interneurons, with particular emphasis on the cases in which GABA receptors are linked to benzodiazepine receptors. We will consider each system in isolation. In Chapter 6 (Section 6.7), we compare the effects of damage to these systems with the effects of anxiolytic drugs and damage to the septo-hippocampal system.

In keeping with the modulatory role suggested by their anatomy (small cell groups diffusely innervating wide areas of the brain), each of these systems has been implicated in psychopathology. We concentrate our efforts most on the systems where the link with anxiety is the strongest.

A10.2 The ascending serotonergic system

The serotonergic systems of the brain have long been thought to be involved in anxiety (see Iversen 1984), as well as in panic, obsessive–compulsive disorder, depression, manic-depressive psychosis, and a variety of other disorders. While many therapeutic agents affect monoamine systems in general, it has recently become clear that specific serotonin uptake inhibitors can be as effective in treating depression, and perhaps anxiety, as the less specific agents (e.g. Moon *et al*. 1994). Our task here will be both to identify the basic functions of the components of the serotonergic system and to isolate their contribution to specifically anxiolytic action. For the latter, we will concentrate largely on serotonergic input to the hippocampus and amygdala; but the data on the role of 5-hydroxytryptamine (5-HT) systems in the control of the hippocampal theta rhythm (Appendix 5) show that other subcortical nuclei, including

probably the locus coeruleus, must also be important targets of serotonergic input involved in anxiety. Similarly, particularly with obsessive–compulsive disorder, serotonergic input to the cingulate cortex is probably important (Appendix 3; but see also Swerdlow 1995).

The most basic experiments in this area employ systemic administration of the serotonin synthesis inhibitor *para*-chlorophenylalanine (PCPA) to deplete whole-brain serotonin. These suggest a role for serotonin in the production of behavioural inhibition, implying an anxiogenic action of the release of this transmitter. PCPA blocks the synthesis of the immediate precursor of serotonin, 5-hydroxytryptophan (5-HTP), by inhibiting tryptophan hydroxylase. Since PCPA could have other pharmacological effects, it is preferable to compare animals treated with this compound with others treated with both PCPA and 5-HTP (which reinstates 5-HT levels without reversing any non-specific effects of PCPA).

PCPA reduces the response suppression produced by punishment (Robichaud and Sledge 1969; Geller and Blum 1970; Wise *et al.* 1973). It has also been reported to alleviate conditioned suppression of an operant response (Hartman and Geller 1971), reduce suppression of bar-pressing when this was put on a Differential Reinforcement of Other behaviour schedule (i.e. reward for anything other than bar-pressing, Thornton and Goudie 1978), and reduce extinction after continuous reinforcement (CRF) training (Beninger and Phillips 1979). These earlier results have been confirmed and extended to the elevated plus-maze, social interaction test, light–dark test, and separation-induced vocalization. Furthermore, where this has been tested, the effects of PCPA have been shown to be reproduced by ventricular injections of the serotonin-specific neurotoxin 5,7-dihydroxytryptamine (see Handley 1995). Suppression of behaviour by punishment has also been reversed by systemic administration of some serotonin receptor blockers (Graeff and Schoenfeld 1970; Winter 1972; Stein *et al*. 1973; Geller *et al*. 1974; Graeff 1974; Cook and Sepinwall 1975). Among the drugs used successfully in this way is methysergide (e.g. Graeff and Schoenfeld 1970; Stein *et al*. 1973).

In iontophoretic experiments methysergide has been shown to antagonize the effects of serotonin in the hippocampus (Segal 1975, 1976) but not in a number of other brain sites (Haigler and Aghajanian 1974). Thus, if methysergide reduces behavioural inhibition by an antiserotonergic action, this could be at serotonergic terminals in the hippocampus. Consistent with this view and with a role for serotonin in anxiety, PCPA does not impair one-way active avoidance, or reduce bar-pressing avoidance of stimulation of the periaqueductal grey.

However, experiments with PCPA and other systemically administered drugs do not always produce such clear-cut results. Thornton and Goudie (1978) were able to block step-down passive avoidance with PCPA, but this effect was only slightly reversed by 5-HTP—sounding a warning about the interpretation of effects of PCPA which have not been challenged with 5-HTP. Blakely and Parker (1973) found no effect of PCPA on punished bar-pressing; and Winocur and Bagchi (1974) found an increased effect of punishment on running in the alley after PCPA. PCPA also appears to increase fear-potentiated startle (an effect which may be incidental to an increase in the baseline startle response; see Handley 1995). More recent experiments have failed to produce more consistent results (see Soubrié 1986 and commentaries).

Mixed effects of serotonin depletion should not surprise us, since we have already found (Appendix 1) that buspirone and other 5-HT_{1A} agonists are anxiolytic. The 5-HT_{1A} agonists will give rise to a net decrease in serotonergic transmission in those 5-HT systems with 5-HT_{1A} autoreceptors, but to a net increase in those systems with 5-HT_{1A} presynaptic or postsynaptic receptors. Since the 5-HT_{1A} drugs are anxiolytic, this implies that a general depletion of 5-HT will have effects that are either 'anxiolytic' or 'anxiogenic' depending on the particular brain site most affected. For example, if release of 5-HT into the amygdala normally acts only on the same 5-HT_{1A} receptors, as does buspirone, then we would expect 5-HT depletion to be 'anxiogenic' and to increase fear-potentiated startle, whereas with raphe neurons (which would normally be suppressed by buspirone acting on autoreceptors), we would expect 5-HT depletion to be 'anxiolytic'.

The effects of PCPA could be interpreted, then, as showing that *systemic* serotonergic antagonists at receptors other than 5-HT_{1A} reduce the suppression of behaviour by punishment and perhaps the omission of reward (but we will see that the latter effect does not seem to be produced by specific serotonergic lesions). Conversely, serotonergic agonists (other than 5-HT_{1A} agonists) directly produce behavioural suppression. This was first shown by Aprison and Ferster (1961) in an experiment in which the levels of serotonin were increased by administration of 5-HTP together with a monoamine oxidase inhibitor to delay the catabolism of serotonin after its release. Similar findings were reported by Graeff and Schoenfeld (1970) and Stein *et al*. (1973) using the long-lasting serotonergic receptor agonist, alpha-methyltryptamine. Similarly, as we discuss in detail below, the anomalous effects of 5-HT_{1A} agonists can be accounted for by their action on autoreceptors, by which they depress serotonergic function.

It should be noted that the reduction in response suppression seen in animals with impaired forebrain serotonergic function is not due to a loss of sensitivity to the primary aversive reinforcer. On the contrary, this is, if anything, increased. The threshold for increased activity or jumping in response to shock is lowered by PCPA, an effect reversed by 5-HTP. The threshold for the detection of shock is unchanged (Harvey and Lints 1971; Fibiger *et al.* 1972; Sheard and Davis 1976).

All these data suggest that serotonin is important for the proper function of brain aversive systems.

A10.2.1 Receptor subtypes

The behavioural role of serotonin systems has been most extensively analysed with systemic injections of putative serotonin receptor blockers. Even recently, there have been only modest numbers of studies using the more satisfactory technique of specific lesion of selected 5-HT pathways with the neurotoxins 5,7- and 5,6-dihydroxytryptamine. More importantly, there have been very few studies which selectively, but totally, deplete serotonin from individual target structures. Care has to be used, therefore, in drawing conclusions about the functional role of serotonin systems. Non-selective depleting agents will affect a range of functionally heterogeneous serotonin systems; and electrolytic lesions or electrical stimulation of a particular serotonergic source nucleus will affect non-serotonergic neurons as well as serotonergic.

Much of the literature uses selective receptor blockers and this might seem to be a satisfactory way of dissecting out specific serotonergic functions. However, there are two problems with this approach. First, the 'selective' drugs only affect *some* of the *collaterals* of any particular serotonergic neuron, and hence will not produce the full effects of inactivation of that neuron. Second, the 'selective' drug will not be selective for any particular serotonergic source nucleus. There is the final complication (noted above) that many, but not all, 5-HT_{1A} receptors are autoreceptors and, as a result, for many sites of action a 5-HT_{1A} receptor agonist is functionally equivalent to a receptor antagonist at one of the other 5-HT receptor subtypes.

Because of the difficulties of interpretation of systemic drug effects, we provide only a very brief discussion of the different receptor subtypes. For extensive reviews see Griebel (1995) and Zifa and Fillion (1992). Where references are not given for statements in the following sections on receptors, they will be found in one of these two reviews (for an additional review see Wilkinson and Dourish 1991).

A10.2.1.1 5-HT_{1A} receptors

The behavioural effects of 5-HT_{1A} agonists were reviewed in Appendix 1. It will be recalled that, as far as they have been tested, their low-dose effects are matched by those of classical anxiolytic drugs, but that in some forms of some tests they are without action. The most important case where they have the same effect as do classical anxiolytics is on animal behaviour that can plausibly be related to the human condition of generalized anxiety disorder. Thus, at least at 5-HT_{1A} receptors, serotonin itself appears to be anxiolytic. As noted above, however, given their role as autoreceptors, this does not mean that serotonin release should be viewed as generally anxiolytic.

A similar distribution of 5-HT_{1A} receptors has been reported in the brains of a wide range of species, including humans. They are most obvious in the septo-hippocampal system (septum, CA1, dentate, entorhinal cortex) and amygdala, where they appear to be postsynaptic, and in the dorsal and median raphe nuclei, where at least 50 per cent appear to be autoreceptors mediating feedback inhibition.

Since 5-HT_{1A} agonists act on raphe autoreceptors, their functional effect on many targets of the ascending serotonin system will be to reduce 5-HT function. The effects of general 5-HT blockade in weakening behavioural inhibition are therefore consistent with the anxiolytic actions of drugs such as buspirone. However, we argue that a significant component of the anxiolytic action of drugs such as buspirone is a result of post- or presynaptic action on non-5-HT cells. The overall effect of the 5-HT_{1A} agonists on behaviour, therefore, involves a combination of functional 5-HT-agonist-like and functional 5-HT-antagonist-like actions.

A10.2.1.2 5-HT_{1B} receptors

These receptors occur in rat and mouse brain, but not in a variety of other species, including man. Their highest density is in the basal ganglia, subiculum, and superior colliculi. They appear to act as terminal autoreceptors, as well as postsynaptic receptors. In human beings the equivalent terminal autoreceptor appears to be 5-HT_{1D}.

As there are no highly selective ligands we will not discuss the possible behavioural role of this receptor here (for a brief discussion see Zifa and Fillion 1992, p. 420).

A10.2.1.3 5-HT_{1C} receptors (5-HT_2 family)

The 5-HT_{1C} receptors have now been reclassified on the basis of their nucleotide sequence as being part of the 5-HT_2 family of receptors and may be referred to as 5-HT_{2C} receptors (in which case 'standard' 5-HT_2 receptors would be referred to as 5-HT_{2A}). They are present at high density in the choroid plexus of a number of species including human and at low levels elsewhere, including the basal ganglia, cerebral cortex, and olfactory tubercles. As there are no highly selective ligands we will not discuss the possible behavioural role of this receptor here (for a brief discussion see Zifa and Fillion 1992, p. 432).

A10.2.1.4 5-HT_{1D} receptors

These receptors have been reported in a very wide range of species, including human. 'In these species, 5-HT_{1B} sites are absent. It has been proposed that 5-HT_{1D} plays the same role in these species as does 5-HT1B in rat, mouse, and opossum. . . . However, it is likely that 5-HT1D sites also exist in the rat brain' (Zifa and Fillion 1992, p. 422). They are very dense in the basal ganglia and less so in the hippocampus, neocortex, and raphe nuclei. They appear to be predominantly postsynaptic. Their role within the brain is unclear, but peripherally they are involved in vasodilation.

A10.2.1.5 5-HT_2 receptors

There is doubt as to whether 5-HT_{2A} and 5-HT_{2B} receptors can be distinguished or whether there is a single receptor with two states. In the present section we conflate these possibilities and refer only to 5-HT_2 receptors. As noted above, these should be distinguished from the 5-HT_{1C} receptor which would now, by some, be referred to as 5-HT_{2C}. The highest densities are located in the hippocampal formation (CA1, CA3, entorhinal cortex) and in closely related areas: frontal cortex, cingulate cortex, nucleus accumbens, hypothalamus, and mammillary bodies. There is also moderate labelling in the basal ganglia.

Of particular interest for the present book is the fact that the mixed 5-HT_2/5-HT_{1C} *antagonist*, ritanserin, can be effective in treating generalized anxiety disorder, despite worsening panic (see Graeff 1993). This mixed action may well match the equally mixed results in tests of animal behaviour, in which 'the drug has been found to produce anxiolytic-like effects in more than 40% of [50] studies while 20% of them reported evidence for increasing anxiety. Finally, 44% of the reports indicated a lack of activity of the drug in these tests. [However] there is no evidence for a greater sensitivity for one or the other category of models. Thus ritanserin has been reported to have disinhibitory effects, anxiogenic-like effects and/or even no effect in the traditional conflict procedures (Geller-Seifter and Vogel), as well as in exploration tests, such as the elevated plus-maze, the light/dark test or the open-field' (Griebel 1995, p. 374). The clinical effects on generalized anxiety could well turn out to show similar unreliability.

5-HT_2 receptors are also involved in the regulation of sleep, temperature, and some aspects of motor control.

A10.2.1.6 5-HT_3 receptors

We have already discussed the 5-HT_3 receptor briefly in our consideration of anxiolytic drugs. However, the highest binding, within the brain, is in the area postrema; this location could account for the well-documented action of the 5-HT_3 antagonist, ondansetron, as an antiemetic in cancer therapy.

Like 5-HT_2 receptors, there are 5-HT_3 receptors in the septo-hippocampal system (septum, hippocampus, entorhinal cortex) and closely related areas: frontal cortex, cingulate cortex, nucleus accumbens, amygdala, thalamus, and hypothalamus. However, 'when compared with other serotonergic receptors, the 5HT3 receptors exhibit a particularly low density in brain (approximately 10 times less in the richest areas)' (Griebel 1995).

Given the theory of this book, this distribution (but not the density) would suggest that ondansetron could be a potential anxiolytic drug, and there is some evidence for this prediction from animal tests, particularly when ethological measures are used. However, there is little evidence of anxiolytic action in conditioning paradigms and, as discussed in Appendix 1, no convincing evidence as yet from clinical studies.

A10.2.1.7 5-HT_4 . . . 5-HT_7 receptors

The number of known types of 5-HT receptors is still increasing rapidly but for 5-HT_n with $n > 3$ there is no useful functional evidence as yet.

A10.2.1.8 5-HT receptors, overview

An extensive review of this complicated area has not been possible within the confines of the present appendix (see Soubrié 1986 and accompanying commentaries; Zifa and Fillion 1992; Griebel 1995; Handley 1995). It is in any case probable that (with the exception of 5-HT_{1A} receptors; see Appendix 1) an extensive review is not desirable, since the different receptor subtypes almost certainly dissect out limited area-specific aspects of a more global function. Given the anatomy of the serotonergic systems, we would expect large areas of the brain concurrently to receive the same serotonergic modulatory signal. It also seems likely that, for the same reasons, specific antagonists will act on separate serotonergic systems which would normally be co-active.

There is considerable commonality in the target sites associated with the different receptor subtypes. 5-HT_{1A}, 5-HT_2, and 5-HT_3 receptors are all found at relatively high densities in the septo-hippocampal system. They are also to be found, but with less consistency, in areas closely associated with the septo-hippocampal system: the amygdala (5-HT_{1A}, 5-HT_3); and the frontal and cingulate cortex, nucleus accumbens, and hypothalamus (5-HT_2, 5-HT_3). With the exception of 5-HT_{1A} and 5-HT_3, serotonin receptors of a wide variety of types are associated with the basal ganglia. The 5-HT_{1A} receptors are also abundant in the dorsal and median raphe nuclei.

It seems likely, therefore, that getting a good separation of receptor subtypes in terms of different functional roles will be very difficult. It also seems probable that the very mixed results of behavioural experiments with the more specific ligands (see Griebel 1995) could be due to quite unphysiological patterns of activation or deactivation of the neural systems normally targeted by a diffuse serotonergic signal.

We move quickly on, therefore, to a more detailed consideration of the anatomy of the serotonergic systems, and ask whether anatomical dissection can enlighten us where pharmacological dissection has largely failed.

A10.2.2 Anatomy of the serotonergic system

The serotonergic systems originate in the raphe nuclei of the brain stem and ascend to the cortex, innervating a wide variety of structures on the way. 'Early studies that used older tracing techniques reported exceedingly few [descending] projections from the dorsal raphe (DR) to the brainstem. . . . [However, there are] moderate to dense projections from the DR [to] pontomesencephalic central gray, mesencephalic reticular nucleus pontis oralis, nucleus pontis caudalis, locus coeruleus, laterodorsal tegmental nucleus, and raphe nuclei, including the central linear nucleus, median raphe nucleus, and raphe pontis' (Vertes and Kocsis 1994, p. 340). There are also extensive projections to the medulla. Of specific interest in the context of defence are the serotonergic inputs to the dorsal and ventral periaqueductal grey, the hypothalamus, the amygdala, and the septo-hippocampal system (see, for example, Handley 1995, Fig. 1). The frontal and cingulate cortices are also important targets.

The serotonergic innervation of the septo-hippocampal system is generally thought to originate mainly in the median raphe (B8 of Dahlström and Fuxe 1964; Bobillier *et al*. 1976; Kellor *et al*. 1977), but with a small contribution from the dorsal raphe as well (B7 of Dahlström and Fuxe 1964; Pasquier and Reinoso-Suarez 1977). It also appears that 'the median raphe supplies the dorsal hippocampus and medial septum, the proposed origins of the Behaviour Inhibition System, while the dorsal raphe nucleus innervates the lateral septum and ventral hippocampus, the possible origin of safety signalling. The amygdala, the 'head nucleus' of the Defence System, is almost entirely supplied by the dorsal raphe nucleus' (Handley 1995, pp. 108–9). However, some recent data suggest that the hippocampus may receive overlapping innervation from the dorsal and median raphe (Hensler *et al*. 1994).

Efferents from the raphe nuclei follow essentially the same three routes as the noradrenergic fibres from the locus coeruleus (Azmitia, in Elliott and Whelan 1978, pp. 80–2; Fig. A10.1; for a recent detailed review see Jacobs and Azmitia 1992) and the cholinergic fibres from the medial septal/diagonal band complex (Gaykema *et al*. 1990). These pathways 'appear to have remained remarkably stable across phylogeny' (Jacobs and Azmitia 1992, p. 179). There is a ventral route which innervates the amygdala en route to the temporal part of the hippocampus. This ventral projection originates in the dorsal raphe. Next there is the septal route. Fibres from the median raphe enter the septal area in the medial forebrain bundle and then pass infracallosally in the fornix–fimbria. The final route is supracallosal in the cingulum bundle. The septal and cingulum bundle inputs innervate the septal parts of the hippocampus (Azmitia and Segal 1978). The median raphe, in addition, innervates the medial septal area and the dorsal part of the lateral septal area. The dorsal raphe innervates the

anteroventral part of the lateral septal area and the nucleus accumbens. Since the dorsal and median raphe innervate the entorhinal cortex, cingulate cortex, and prefrontal cortex, there is serotonergic input (as there is noradrenergic input) of some type to all parts of the septo-hippocampal system and its major cortical output areas. As we will see, the topographic differentiation of this system is mirrored by that of the cholinergic input to the septo-hippocampal system.

Fig. A10.1 [plate for this figure to be recovered from Figure 3.12 of the first edition]

Fig. A10.1 The raphe nuclei in the brain stem of the rat as shown by an immunohistofluorescent technique. rd, dorsal raphe (B 7); ncs, nucleus centralis superior or median raphe (B 8); LM, lemniscus medialis (B 9); AC, cerebral aqueduct. Bar: 50 μm. For abbreviations in the schematic diagram of the brain region to which the photograph corresponds, see Steinbusch (1981), from which the figure is taken.

The relative projections of the dorsal and median raphe to different structures are summarized in Fig. 6.6 (see also Handley 1995, Fig. 1). Of particular interest for our present purposes, the dorsal raphe sends projections (which may well be collaterals of the same source cells and hence carry the same information} to the periaqueductal grey (5-HT_2/5-HT_{1C} receptors; this projection is not emphasized in either figure), the amygdala (5-HT_{1A} receptors), the ventral striatum (5-HT_{1D} receptors), the hippocampus (5-HT_{1A} receptors), and the frontal cortex (5-HT_2 receptors). All of these structures are thought to have some role in different aspects of defensive behaviour.

The dorsal raphe is also ‘thought to play a role in modulating circadian rhythms . . . [and] these modulations may be, in part, mediated by the [direct] retinal projection to the periaqueductal gray and serotonin neurons in the dorsal raphe nucleus’ (Shen and Semba 1994, p. 166; see also review by Morin 1994).

A10.2.3 Dorsal and median raphe lesion/injection

For the reasons given earlier, we will review here only those cases in which the raphe nuclei have been lesioned with a specific serotonergic neurotoxin, or where a drug has been injected directly into a nucleus. Where no reference is given, the citations can be found in Griebel (1995, Table 1) or Handley (1995, Table 1).

Neurotoxic lesions of the dorsal combined with the median raphe have been shown to reduce activity and rearing in the open field, reduce social interaction (and locomotion), have no effect on drinking in a novel environment, and have no effect on aggression to an intruder. They also appear to produce a selective release of punished responding which is not accompanied by a general release of non-rewarded responding (Tye *et al.* 1977), although they do impair acquisition of a differential reinforcement of low rates of response 20-s (DRL-20) schedule, probably because of the median raphe component of the lesion (Fletcher 1995). They also improve acquisition of a delayed conditional temporal discrimination (Al-Zahrani *et al*. 1996).

A10.2.4 Dorsal raphe lesion/injection

Specific dorsal raphe lesions have been shown to increase responding during acquisition of the Geller–Seifter schedule and Vogel conflict test, to increase social interaction in the high-light/unfamiliar component of the social interaction test, and to produce anxiolytic effects in the elevated plus-maze. In all of these cases null or opposite effects have also been reported.

Injection of 5-HT or 5-HT_{1A} agonists into the dorsal raphe would be expected to have effects equivalent to neurotoxic lesions because of an action on autoreceptors. Such injections have been shown to have anxiolytic-like effects in the Geller–Seifter test, Vogel test, social interaction test, light–dark box, conditioned emotional response, and with periaqueductal grey stimulation. They do not (unlike dorsal raphe muscimol or median raphe 5-HT_{1A} agonists) release responding on a DRL-20 schedule (Fletcher 1994), and appear to have no effect on open-field exploration. While affecting inhibitory avoidance, they do not affect one-way escape (Graeff *et al*. 1996*a*). They produce a selective increase in alcohol as opposed to water consumption (Tomkins *et al.* 1994); block the effects of exposure to inescapable shock (Maier *et al.* 1995*a*); produce feeding in non-deprived rats (see Fletcher 1991); and, at least at modest doses, can act as a reinforcer in a conditioned place preference task (Fletcher *et al.* 1993). These latter effects are likely to be mediated via the release of dopamine into the accumbens and caudate resulting from a loss of 5-HT-mediated inhibition (Fletcher 1991).

Injection into the dorsal raphe nucleus of a benzodiazepine inverse agonist (which would be expected to produce a net activation of the nucleus) or of the excitatory amino acid, kainic acid, facilitated inhibitory avoidance in an elevated T-maze test, while at the same time failing to affect, or even decreasing, active avoidance in the same apparatus (Graeff *et al.*, in press). Treatment of this kind also produces effects similar to those of inescapable shock (Maier *et al*. 1995*b*). Kainate had no effect on ambulation or rearing in an open field.

Overall, then, the release of 5-HT by activation of the dorsal raphe would appear to be anxiogenic in tests of conditioned punishment, conditioned emotional response, social interaction, and the elevated plus-maze, but appears to lack anxiogenic action in less stressful tests (the only case so far tested being open-field exploration and rearing). Consistent with the opposite direction of effect of systemic PCPA, release of dorsal raphe serotonin appears to increase passive avoidance specifically and may decrease active avoidance.

Thiébot *et al*. (1984) report that neurotoxic lesions of the dorsal raphe do not change the response-releasing effects of benzodiazepines. However, they found no effect of the 5,7 di-hydroxytryptamine lesions themselves (unlike a number of other studies) and their depletion of serotonin was less than 80 per cent. This result also contrasts with an earlier report in which these authors found that chlordiazepoxide injected into the dorsal raphe released suppression (Thiébot *et al.* 1980). However, depletion of 5-HT with PCPA, while disinhibiting punished behaviour, does not interact with the response-releasing effects of midazolam (Plaznik *et al*. 1994). Similar complications are found with the anxiogenic effect of inescapable shock. Benzodiazepine injections into the dorsal raphe are anxiolytic in this situation when systemic injections are not. This lack of effect of systemic benzodiazepines can be accounted for if the drugs have two effectively opposing actions at the level of the periaqueductal grey. On the one

hand, the benzodiazepines can increase GABAergic inhibition on the raphe (as shown by the effects of intra-raphe microinjection), reducing its output, so releasing activity in the periaqueductal grey and reducing 'anxiety' as measured in the inescapable shock paradigm. On the other hand, with systemic benzodiazepine injections, GABA activity would be enhanced also directly in the periaqueductal grey, dampening its output and so reversing the effects of the raphe-mediated changes (Maier *et al.* 1994). We discuss these results further when we consider interactions between the aminergic systems.

Cooling of the dorsal raphe or injection there of hypnogenic peptides induces sleep. However, this effect is not produced by injection of 5-HT_{1A} agonists (El Kafi *et al.* 1994, p. 220), suggesting an additional involvement of non-5-HT mechanisms.

A10.2.5 Median raphe lesion/injection

As with dorsal raphe lesions, we will review here only those (unfortunately very few) cases where the median raphe has been lesioned with a specific serotonergic neurotoxin, or where a drug has been injected directly into the median raphe. Where no reference is given, the citations can be found in Griebel (1995, Table 1) or Handley (1995, Table 1).

Specific median raphe lesions have been shown to have no effect in the hole-board test or on social interaction, but they do impair acquisition of a DRL-20 schedule (Fletcher 1995). Injections of 5-HT or 5-HT_{1A} agonists into the median raphe (which should have functional effects equivalent to those of a lesion (e.g. Bosker *et al.* 1994) have been shown to be anxiolytic in Vogel's conflict test and in the light–dark test, as well as on a DRL-20 schedule (Fletcher 1994). In the latter case the effect was notably smaller, and possibly qualitatively different, from the effects of muscimol (a $GABA_A$ antagonist) injection into the same area, suggesting that both serotonergic and non-serotonergic neurons contribute to this behaviour. Consistent with this anxiolytic effect, 5-HT_{1A} agonist injection into the median raphe can act as a reinforcer in a conditioned place preference task (Fletcher *et al.* 1993). This treatment also induces feeding in non-deprived rats through a release of dopamine into the accumbens and caudate nuclei consequent on the loss of serotonergic inhibition (Fletcher 1991).

'Intra-median raphe injections of the GABA-A agonist muscimol . . . result in very pronounced hyperactivity and in robust increases in food and water intake by non-deprived animals. . . . [However], neither the hyperactivity and increased ingestive behaviour nor the increases in dopamine turnover produced by muscimol appear to be dependent on intact serotonergic mechanisms. The simplest explanation of these findings is that GABA-A receptors are found both on serotonergic and non-serotonergic neurons within the median raphe and that inhibition of the non-serotonergic cells plays a preeminent role in mediating the behavioural effects of muscimol injections'. Similar results are obtained with the $GABA_B$ agonist baclofen (Wirtshafter *et al.* 1993, p. 83) and with mu and delta, but not kappa, opioid agonists (Klitenick and Wirtshaffer 1995), which, like GABA, inhibit raphe neurons (Alojado *et al.* 1994).

Clearly, further work is required, especially in the cases where no effect has been reported; however, on the present evidence it appears that median (like dorsal) raphe release of 5-HT is anxiogenic in operant conflict tests (one example) and perhaps with some innate responses (one positive case, but two negative). Thus, on the evidence so far, there is no great reason to separate the functions of the dorsal and median raphe (but see, for example, Deakin and Graeff 1991).

A10.2.6 Injections of drugs into targets of serotonin afferents

Intra-amygdala injection of 5-HT is anxiogenic in the Geller–Seifter test and social interaction test. Injection into the amygdala of 5-HT_{1A} agonists is anxiogenic in the Geller–Seifter test, while injection of the non-specific antagonist methysergide is anxiolytic. These data are all consistent with an anxiogenic role for serotonin in the amygdala, but contrast with the anxiolytic effects of systemic administration of 5-HT_{1A} agonists and of the partial agonist buspirone injected into the amygdala on fear-potentiated startle.

Injection of the 5-HT_{1A} agonist 8-OH-DPAT into the hippocampus is anxiolytic in the Vogel conflict test (as is the benzodiazepine midazolam; Stefanski *et al*. 1993), the open field and the elevated plus-maze, but not defensive burying—this last pair providing a double dissociation from the effects of septal injections (Menard and Treit 1998). However, injection of buspirone into the hippocampus is anxiogenic or ineffective in the Vogel test; and, complicating matters still further, anxiolytic in the elevated plus-maze and open field.

Injection of 8-OH-DPAT into the nucleus accumbens is anxiolytic in the Vogel conflict test and the open field. However, as with hippocampal injections, intra-accumbens buspirone is anxiogenic in the Vogel test. Injection of 8-OH-DPAT into the septum is ineffective in the plus-maze but anxiolytic on defensive burying.

Given the U-shaped dose–response curves of 5-HT_{1A} agonists when delivered systemically, it is perhaps not surprising that we should get both anxiolytic and anxiogenic effects from the same drug administered to different brain sites. It may seem more surprising that different 5-HT_{1A} agonists should have different effects in the same brain site. However, some of the drugs used are only partial agonists and there could be major differences in their effects as a result of differing receptor affinities and receptor numbers in the different regions. Nonetheless, it is surprising that buspirone, the most conclusively anxiolytic of the 5-HT_{1A} agonists in clinical tests, should be so consistently anxiogenic at the 5-HT terminal sites tested. As we point out when we discuss the possible role of the hippocampal formation in the actions of buspirone, it may simply be that the predominant site (or sites) of action of sytemically administered buspirone have not been tested; and that the effects observed with intra-hippocampal injection are a consequence of negative feedback mechanisms that are activated only when very high concentrations of 5-HT (or a 5-HT agonist) are released onto the receptors.

A10.2.7 Raphe cell firing

There have only been a modest number of studies of the firing of single cells in the raphe in freely moving animals, mostly in the dorsal raphe (for a review of single cell

studies in anaesthetized animals see Jacobs and Azmitia 1992, pp. 194–8). Stressors such as white noise, restraint, and confrontation with a predator (all of which increase firing of locus coeruleus cells) have little effect on dorsal raphe firing. However, dorsal raphe firing 'increases monotonically as an animal moves from REM sleep, through the stages of slow wave sleep, to quiet waking, and finally to an active waking state' (Wilkinson and Jacobs 1988, p. 446), as does the release of 5-HT in the nucleus (Portas and McCarley 1994). Nonetheless, it appears that anaesthesia can 'reveal' otherwise quiescent dorsal raphe neurons (Montagne-Cavel *et al.* 1995). Consistent with the relative lack of effect of long-duration stressors, noxious heat stimulation produced little or no effect even in neurons which responded to light touch or pinch stimuli. Responses to light touch and pinch were found in only about 50 per cent of neurons (Montagne-Cavel *et al.* 1995). Thus, 'the raphe groups of serotonergic neurons are not primarily involved in [specific behavioural] activities, since no effect on serotonergic single-unit activity in behaving animals was seen independent of changes in [general] behavioural arousal. This was true despite the fact that . . . stimuli [were] chosen explicitly for the strength of their impact on the organism' (Jacobs and Azmitia 1992, p. 209). Unfortunately, no single-cell recording studies have used the specific behavioural paradigms in which the lesion results suggest that the dorsal raphe is involved.

An interesting point is that 'serotonin neurons have a characteristic discharge pattern that distinguishes them from most other cells in the brain. They are relatively regular, exhibiting a slow and steady generation of spikes. Serotonin neurons retain this rhythmic pattern even if they are removed from the brain and isolated in a dish' (Jacobs 1994, p. 459). Furthermore, 'the message . . . from serotonergic neurons may be an analog signal that indicates that the organism is in REM sleep (~0.0–0.3 spikes/s), in slow wave sleep (~0.3–1.5 spikes/s), drowsy (~1.5–2.0 spikes/s), in quiet waking (~2.0–3.0 spikes/s), active waking (~3.0–5.0 spikes/s), or physically aroused (~5.0–7.0 spikes/s)' (Jacobs and Azmitia 1992, p. 209). The fact that these neurons are briefly depressed during orienting and increase their activity with repetitive movement (often phase locked to the movement) led Jacobs (1994, p. 461) 'to conclude that the primary function of the brain serotonin system is to prime and facilitate gross motor output in both tonic and repetitive modes.'

Consistent with the suggestion that 5-HT_{1A} receptors in the dorsal raphe are autoreceptors, systemic administration of 5-HT_{1A} agonists depresses dorsal raphe firing (Matheson *et al*. 1994), as does iontophoresis into the dorsal raphe itself (Vandermaelen *et al*. 1986). However, in some cases it appears that the depression of raphe neurons is indirectly mediated, at least at low doses, by the frontal cortex (Ceci *et al.* 1994).

A10.2.8 Overview

An integration of the role of 5-HT systems, at least with respect to defensive behaviour, has recently been offered by Graeff (1993; see also Graeff *et al*. 1996*a*). Here, we briefly summarize Graeff's conclusions; their main support and references will be found in his paper.

Graeff approaches the functions of the serotonin systems from the point of view of the essentially hierarchical organization of defensive systems, discussed at the ethological

level in Chapter 2 and with respect to the dorsal periaqueductal grey–hypothalamic–amygdalar system in Chapter 6.

In Chapter 6 we concluded that the dorsal periaqueductal grey and hypothalamus coordinate responses to an immediate predator (freezing, fight, flight, autonomic discharge, analgesia). We also concluded that the amygdala is concerned with active avoidance and learned escape, that is with behaviour controlled by CS-Pun– (see Chapter 3 for definitions of this and similar terms). It appeared that the amygdala discharges these functions by receiving direct input from the ascending sensory systems and also the parahippocampal and possibly hippocampal areas; and, through the process of long-term potentiation or some other form of plasticity, attaches these inputs to the relevant motor programmes for avoidance.

In many circumstances both the escape and the avoidance system will be concurrently excited by sensory stimuli. For example, at some point during learning of escape the dorsal periaqueductal grey will be activated by Pun (i.e. shock) stimuli at the same time that the amygdala is activated by CS-Pun–. This coactivation could, in principle, be disentangled by input from the dorsal raphe. The release of serotonin from the terminals of dorsal raphe neurons increases defensive reactions mediated by the amygdala, but decreases defensive reactions mediated by the dorsal periaqueductal grey and probably the hypothalamus (see Deakin and Graeff 1991). Firing of dorsal raphe neurons, therefore, will shift the balance from a high probability of undirected escape behaviour to a high probability of directed avoidance. Since the amygdala is involved in responses to both CS-Pun– and CS-Pun+, the signal from the dorsal raphe could possibly be characterized as simply CS-Pun (with the implication that the punishment is avoidable in some way), but we argue that this signal is in fact much broader.

Graeff himself suggests ‘that 5-HT facilitates defensive behaviour elicited by potential or distal danger signals . . . by acting on the amygdala, but, in the periventricular system, inhibits the expression of fight/flight responses that are adaptive only when the threat stimulus is proximal to the animal.’ These conclusions make sense of many of the conflicting results in tasks which could involve concurrent activation by threatening stimuli of both the amygdalar and dorsal periaqueductal grey systems: the same direction of change in 5-HT release should have opposite effects on behaviours mediated by these two systems (see also Graeff *et al.* 1997), and on occasion the net effect could be an apparent lack of change.

An apparent exception to this facilitation of amygdalar defence mechanisms by 5-HT (as with a number of other points already considered) is fear-potentiated startle. Treatment with PCPA increases, rather than decreases, potentiated startle (Davis *et al*. 1988). As we noted earlier, this effect of PCPA is consistent with that of the 5-HT_{1A} agonist buspirone on fear-potentiated startle. In this respect the effects of 5-HT agonists might make fear-potentiated startle, despite its general sensitivity to anxiolytics (with the notable exception of imipramine), seem better classified with active avoidance and escape behaviours than with the passive avoidance and behavioural inhibition we have associated, theoretically, with anxiety.

However, the output of the serotonergic system is very widespread, and the behavioural correlates of single-cell firing in the raphe nuclei very broad. By focusing

on the role played by serotonergic transmission in the behavioural inhibition system, we may be forcing it into too tight a mould. Consider, therefore, Jacobs's suggestion (see section on firing of raphe cells, above): that serotonin release primes motor systems concerned with tonic or repetitive action, while orienting responses (and by implication other sudden reactions, including output from the fight–flight system) are accompanied by suppression of serotonin release. On this view, the effects of serotonin release in tests of emotional beahviour might be accounted for by the fact that they involve different modes of operation of motor systems. Release of serotonin would not, then, necessarily be associated with any specific emotional state. The importance of serotonin for emotion and emotional disorder would stem from the fact that emotion often requires directed action.

We concluded that the amygdala mediates both active and passive avoidance (Chapter 6), but that the septo-hippocampal system is involved in passive but not active avoidance (Appendix 8). Injection of 5-HT or 5-HT_{1A} agonists into the amygdala increases response suppression. Similarly, activation of the dorsal raphe facilitates inhibitory avoidance in the elevated T-maze (Graeff *et al.* 1996*b*). However, active avoidance is not decreased by PCPA, which should lower 5-HT levels in the amygdala, nor increased (and even decreased) by dorsal raphe activation (Graeff *et al.* 1996*b*). The effects of 5-HT, then, including those mediated in the amygdala, appear to be specific to passive as opposed to active avoidance. This behavioural specificity, coupled with the fact that the systemic effects of the 5-HT_{1A} agonist buspirone are anxiolytic (in contrast to the anxiogenic effects of 5-HT_{1A} agonists injected directly into the amygdala), suggests a role for the septo-hippocampal system in mediating some of the effects of raphe activation, in addition to those mediated by the amygdala and periaqueductal grey.

The 5-HT pathways to the hippocampus originate in both the dorsal and the median raphe. They innervate both the septal area and hippocampus extensively but diffusely. Stimulation of the raphe nuclei facilitates the passage of information round the hippocampal circuit, an effect that is blocked by antiserotonergic drugs (Segal 1975; Assaf and Miller 1978). This effect is similar to that seen after locus coeruleus stimulation (see below). As far as the dorsal raphe is concerned, this facilitatory effect on neuronal transmission in the septum and hippocampus matches the facilitatory effect of 5-HT release on amygdalar function, noted above, but contrasts with the inhibitory effect of 5-HT release on the function of the dorsal periaqueductal grey. In contrast to its effects in the hippocampus proper, serotonin appears to be inhibitory in the entorhinal (Schmitz *et al*. 1995) and prefrontal cortex (Read *et al.* 1994). The 5-HT system has complex effects on the control of hippocampal theta rhythm (Appendix 5), which appear to result from opposing effects of 5-HT on functionally related centres, as seen also in the case of the interactions between the amygdala and periaqueductal grey.

We can, thus, integrate much of the available data with the view that the release of serotonin (and the firing of raphe cells) is related to the priming of tonic or repetitive motor circuits and the concurrent inhibition of phasic, orienting, startle, and related circuits. This priming also has major effects on circuits (primarily in the septo-hippocampal system) whose business is to *inhibit* ongoing, tonic motor circuits. Thus, the firing of serotonin cells will often be functionally silent; and the effects of serotonin on behavioural inhibition, for example, will become functionally evident

only when other conditions are fulfilled. One way to look at this is to view the serotonin signal as increasing 'motor attention', an effect that will have obvious behavioural consequences only when an event occurs to interrupt the motor programme. (This notion of behaviourally silent output from the raphe serotonin system is extended in the discussion of the control of hippocampal theta rhythm in Appendix 5.)

A10.3 The dorsal ascending noradrenergic bundle

The noradrenergic systems have been no more exclusively linked to anxiety than serotonergic ones. Drugs that block the beta noradrenergic receptor ('beta-blockers') have been used particularly in treating performance anxiety and post-traumatic stress disorder, but their therapeutic action appears to be largely peripheral. As with serotonin, noradrenaline appears to have an important role in stress and depression, and 'the expression of tyrosine hydroxylase [the rate-limiting enzyme in the synthesis of noradrenaline; see below] in locus coeruleus may be relevant in the pathophysiology of suicide' (Ordway *et al.* 1994, p. 680).

A10.3.1 Anatomy of the ascending noradrenergic system

As a potential site for the action of anxiolytic drugs on defence systems, the ascending noradrenergic system no less attractive than the ascending serotonergic system. The ascending noradrenergic system arising in the locus coeruleus shares many of the anatomical and physiological properties of the raphe serotonin system. The noradrenergic pathway of most interest in the present context originates in the locus coeruleus and ascends in the dorsal noradrenergic bundle (DANB) to innervate much of the forebrain (Fig. 6.5), including the frontal cortex, cingulate cortex, pyriform cortex, hippocampal formation, amygdala, thalamus, hypothalamus, and basal forebrain. There are GABAergic terminals in the locus coeruleus on which the classical anxiolytics can act (Iversen and Schon 1973). However, the action of benzodiazepines on evoked as opposed to spontaneous activity in the locus coeruleus is mediated elsewhere (Simson and Weiss 1989). The amygdala, septum, and hippocampus are all major targets for the dorsal bundle efferents from the locus coeruleus which could mediate effects on anxiety. The ventral noradrenergic bundle runs from the locus coeruleus (and other noradrenergic nuclei) to provide additional innervation of areas such as the hypothalamus, but has not been as extensively studied as the dorsal bundle.

To reach the hippocampus, the dorsal bundle (like the serotonergic and cholinergic innervation) splits into three parts. The dorsal part passes through the septum to course over the corpus callosum, in the cingulum bundle, entering the hippocampus over the splenium of the corpus callosum. This gives rise to afferents to the frontal and cingulate cortex *en passage*. The medial part passes through the septum, innervating the medial and lateral septal nuclei, before reaching the hippocampus via the fornix–fimbria. The ventral part passes through the ventral amygdaloid bundle, innervates the amygdala and pyriform cortex *en passage* and then innervates the temporal (ventral) parts of the hippocampus.

The locus coeruleus is the sole source of noradrenergic innervation of the hippocampal formation, but only provides about 50 per cent of the noradrenergic innervation of the medial and lateral septum (Owen *et al*. 1982). Areas A1 and A2 in the medulla oblongata (Fig. 6.5) send fibres in the ventral noradrenergic bundle which provide the other 50 per cent (Björklund, in Elliott and Whelan 1978, p. 127; Moore and Bloom 1979).

The locus coeruleus may receive some feedback from the hippocampal formation. This appears to be largely from the temporal (ventral) portion of the subiculum (Swanson 1978). According to Swanson, the connections of this area (see Appendix 4) suggest that it is more closely related to the amygdala than the rest of the septo-hippocampal system, and it may be significant that the projection from areas CA3 and CA4 to the entorhinal cortex is also predominantly from the temporal part of the hippocampus.

The anatomy of the locus coeruleus and the DANB place severe constraints on the functions which can be plausibly attributed to them (Ungerstedt 1971; Lindvall and Björklund 1978; Moore and Bloom 1979). There are only about 1500 cells in the locus coeruleus of the rat. This minuscule number of cells innervates widespread regions of the brain, including the olfactory bulb, much of the neocortex, the hippocampus, septal area and amygdala, some thalamic and hypothalamic nuclei, the geniculate bodies, the cerebellum, and the spinal cord. To achieve this feat, each cell body gives rise to several bifurcating axons (Olson and Fuxe 1971; Pickel *et al.* 1973). These observations seem to exclude the possibility that the DANB conveys any detailed or highly patterned information.

There is some modest differentiation within the locus coeruleus in terms of which terminal areas are innervated by which groups of cells within it (Mason and Fibiger 1979*a*). McNaughton and Mason (1980) suggest that this element of specificity can perhaps be enhanced by the action of recurrent collaterals, which could modulate the firing of adjacent cells (Aghajanian *et al*. 1977; Shimizu *et al*. 1978; Watabe and Satoh 1979). However, each individual cell appears to have many projection sites, and it remains very unlikely that the DANB carries as precise or detailed information as, say, a primary sensory pathway. This view is reinforced by the fact that, within any target structure, the locus coeruleus has a diffuse and ramifying pattern of terminal projections and, with the exception of the dentate gyrus of the hippocampus (Koda *et al*. 1978), its nerve endings are non-specialized, suggesting a neurohormonal or neuromodulatory role (Descarries *et al*. 1977; Shimizu *et al*. 1979).

The afferents to the locus coeruleus do not provide us with any clearer picture of its function (Luppi *et al*. 1995). It receives input from infralimbic, insular, and frontal cortex; from several components of the posterior hypothalamus; from the periaqueductal grey and mesencephalic reticular formation; from the raphe nuclei; from the pontine reticular formation, laterodorsal tegmental nucleus, Kölliker–Fuse nucleus, lateral parabrachial nucleus and from A5. The input from the frontal cortex appears to be inhibitory (Sara and Hervé-Minvielle 1995). Within the medulla the primary inputs to the locus coeruleus seem to be from the lateral paragigantocellular nucleus and the dorsomedial rostral medulla. These inputs form no coherent pattern with respect to the defence system as so far delineated (there is little or no input from the amygdala), nor do they provide us with any clear alternative. As we will see,

coherence can be inferred, but only once we have considered the lesion and single-cell recording data.

A10.3.2 The behavioural effects of DANB lesions

As noted earlier, it is difficult to infer function from correlations between brain activity and behaviour, but these problems can be overcome to some extent by comparison with the effects of lesions. In the case of the DANB, almost total lesions can be produced, in the absence of damage to adjacent structures, by injection of the catecholamine-specific neurotoxin 6-hydroxydopamine.

In the first edition of this book (pp. 320–4) we considered some of the advantages and disadvantages of this technique. We concluded that it is best to infer DANB function only from the effects of neurotoxic lesions; that a lack of effect of a neurotoxic DANB lesion cannot be taken at face value if noradrenaline in the hippocampus is reduced by less than 90 per cent; and that, in some cases at least, the adrenal gland is able to substitute for a lesioned DANB, that is, a behavioural deficit is seen only if both the DANB and adrenals are lesioned.

We shall consider the data on DANB lesions within the same general framework used in Appendix 1 to encompass the effects of anxiolytic drugs. Our conclusions are summarized in Table 4.2 of the printed text. Where no justification is given here for the contents of this table, it can be found in the first edition (pp. 324–46; see also reviews by Mason and Iversen 1975; McNaughton and Mason 1980).

A10.3.2.1 Responses elicited by appetitive and aversive stimuli, rewarded behaviour, and responses elicited by non-reward

Although responses elicited by appetitive stimuli have not been specifically studied, since rewarded behaviour is intact after DANB lesions, there is no reason to suppose that they would be affected. Neither active responses elicited by shock nor pain thresholds are affected by DANB lesions. Reduced freezing during a session in which responses to shock were measured (Mason and Fibiger 1979*b*) matches that seen in the septo-hippocampal syndrome (Appendix 8).

Ögren and Fuxe (1974) found that combined dorsal bundle lesions and adrenalectomy elevated the pain threshold in the hotplate test. This finding may account for the impaired escape, one-way and two-way active avoidance and passive avoidance seen after the combined lesion (Ögren and Fuxe 1974, 1977; Wendlandt and File 1979).

DANB lesions alone do not impair learning of a variety of rewarded responses. Nor do they change the frustration effect in the double runway (Owen 1979; Owen *et al*. 1982). They do not appear to affect a variety of simultaneous discriminations.

A10.3.2.2 One-way active avoidance and escape

DANB lesions do not, by themselves, impair escape or active avoidance responses. However, addition of adrenalectomy produces an impairment (Ögren and Fuxe 1974, 1977). This combined treatment also affects pain thresholds. This, together with the

fact that escape and active avoidance are not sensitive to anxiolytic drugs or septo-hippocampal lesions suggests that this result is not relevant to the noradrenergic contribution to septo-hippocampal function. It appears, however, that the defence system as a whole requires intact input from either the locus coeruleus or the adrenals.

A10.3.2.3 Classical conditioning of fear

DANB lesions do not reliably affect off-the-baseline conditioned suppression, but there are indications that, like anxiolytic drugs and septo-hippocampal lesions, they can affect on-the-baseline conditioned suppression (Tsaltas *et al.* 1989; but see Lorden *et al*. 1979). In different experiments they have been reported to reduce conditioning of fear to explicit stimuli, while increasing conditioning of fear to background stimuli (Selden *et al.* 1990); reduce explicit conditioning, while leaving conditioning to an explicit context stimulus untouched (Tsaltas *et al*. 1989); or even *increase* conditioning of fear to both types of stimuli (Selden *et al.* 1991).

Selden *et al*. (1991) review a range of studies which obtain a range of effects of this type, and conclude that 'the one variable which appears to account for the various effects of ceruleo-cortical NA depletion on conditioning to explicit stimuli is the specific temporal contingency between CS and US occurrence' (Selden *et al*. 1991, p. 153). We discuss the possible role of temporal interval in conditioning in some detail in Chapter 8 of the printed text and conclude that this parameter will usually be confounded with other less tangible entities such as interference. That this may be a problem here also is indicated by the fact that 'the strongest evidence contradicting the above generalization is a series of 3 experiments, reported by Cole and Robbins, which used a 0-s trace interval procedure and found consistent impairments in CS conditioning in DANB-lesioned rats. However, in these experiments, but in none of the other studies reported here, the lesioned rats also exhibited increased levels of pre-CS responding' (Selden *et al*. 1991, p. 152).

A10.3.2.4 Passive avoidance, two-way active avoidance, non-spatial active avoidance

The data on passive avoidance are complex (see first edition pp. 327–31) and, unfortunately, few. It is clear that DANB lesions can produce a passive avoidance deficit under some conditions. The data are not inconsistent with the idea that the DANB lesion produces a weaker form of the septo-hippocampal deficit. This hypothesis predicts the strongest effects in tasks in which punishment conflicts with locomotion towards a reward.

Perhaps more surprisingly, the data on two-way active avoidance are also inconsistent. This task is reliably improved by anxiolytic drugs and septal and hippocampal lesions. However, it is impaired by amygdala lesions. There are some reports of improvement with DANB lesions (Ögren and Fuxe 1977; Mason and Fibiger 1979*b*), but also one of no change (Wendlandt and File 1979). There is also one report in which DANB lesions failed to impair Sidman avoidance (Mason and Fibiger 1979*c*). It may be that these conflicting results are the consequence of simultaneous but opposing actions of noradrenaline in the amygdala and the septo-hippocampal system.

A10.3.2.5 Reward omission and successive discrimination

Increased resistance to extinction of a previously rewarded response is the most consistent and well-replicated finding with dorsal bundle lesions. It has been obtained in a wide variety of apparatus and also after a variety of reward schedules. (Increased resistance to extinction is also seen with aversively motivated tasks.) The main exception is with overtraining. With 100 acquisition trials, no increase in resistance to extinction is obtained (Koob *et al*. 1978; Owen *et al*. 1979, 1982).

Given this result, and the implied extinction component in reversal learning experiments, it is surprising that DANB lesions have no effects on reversal of a T-maze position habit (Roberts *et al*. 1976) or of a ball-pushing task (Mason and Iversen 1977).

An even more puzzling discrepancy is that DANB lesions impair conventional successive discrimination, as we would expect from the results with anxiolytic drugs and septo-hippocampal lesions, but do not impair performance on a DRL schedule. In this context, it is interesting that an equivalent dissociation is obtained with respect to the mechanism of action of benzodiazepines. The opiate antagonist naloxone blocks the effects of benzodiazepines on DRL, but does not do so with successive discrimination (Tripp and McNaughton 1987, 1992; Tripp *et al.* 1987).

Salmon *et al.* (1988) found that the impairment in successive discrimination did not occur if a steady light signalled the rewarded (in this case, on a variable interval, VI, schedule) phase and a flashing light signalled non-reward, but did occur with the (easier) discrimination when the significance of the stimuli was reversed. This result is inconsistent with a simple attentional hypothesis of DANB function, but is consistent with the idea that the lesions reduced behavioural inhibition (see discussion in Salmon *et al*. 1988).

The effects of DANB lesions on fixed interval (FI) responding are variable, and when there are effects (Owen 1979) this involves increased responding at the end, but not the beginning, of the FI interval. This may be related to the fact that naloxone blocks the effects of a benzodiazepine only in the initial part of the FI and not in later parts (Tripp and McNaughton 1992).

A10.3.2.6 Maze learning

Few experiments have investigated maze learning after DANB lesions, but there are indications of a restricted impairment. Left–right alternation in the T-maze is impaired (Mason and Fibiger 1978*b*); and, with intracerebral 6-hydroxydopamine rather than specific DANB lesions, Leconte and Hennevin (1981) found impaired performance in a multiple T-maze. DANB lesions also block spontaneous alternation (McNaughton *et al*. 1984).

In their experiment using the multiple T-maze, Leconte and Hennevin (1981) also measured the duration of slow wave and rapid eye movement (REM) sleep in the period immediately after the daily learning session. It is well established that, during the time when learning is proceeding fastest, there is a brief augmentation of REM sleep shortly after the end of each training session (Bloch *et al.* 1978). Leconte and

Hennevin (1981) report the striking finding that their lesion eliminated both the accelerated phase of the learning curve (speed of traversal of the maze) and the augmentation of REM sleep which, in the controls, accompanied this phase. These results, if they are due to changes in the DANB, are consistent with the observations of Kovacs *et al*. (1979) and Zornetzer and Gold (1976), who showed increased sensitivity to DANB lesions at longer retention intervals with step-through passive avoidance. In each case the experimental observations suggest that the DANB plays some role in strengthening the effects of at least some learning experiences.

DANB lesions do not usually affect acquisition in the Morris water maze (but see below).

A10.3.2.7 Responses elicited by novelty, habituation

Neither overall activity level nor locomotion in a novel environment is generally affected by DANB lesions. Rearing in the open field, however, is reduced by damage to the DANB (Leconte and Hennevin 1981; McNaughton *et al.* 1984), especially if it is combined with adrenalectomy (Ögren and Fuxe 1974; Wendlandt and File 1979).

In the T-maze, DANB lesions eliminate not only spontaneous alternation (see previous section) but also the response to stimulus change (McNaughton *et al*. 1984). However, the time spent in contact with a novel object is increased (Mason and Fibiger 1977; Mason *et al.* 1978), while exploration of a hole-board is unaffected (Crow *et al*. 1978; Wendlandt and File 1979).

Crow *et al*. (1978) also demonstrated that DANB lesions are without effect in File's (1980) social interaction test.

Habituation is not affected by DANB lesions in a variety of different situations. Habituation of distraction in the alley has been reported to be both impaired (Koob *et al*. 1978) and improved (Fibiger *et al*. 1975). The increased contact with a novel object noted above could be interpreted as a loss of habituation; but this seems unlikely, given the lack of any other clear case.

A10.3.2.8 Fearful behaviour

Neither defecation nor grooming in the open field is affected by dorsal bundle lesions (Kovacs *et al*. 1979; Wendlandt and File 1979). This suggests that fear produced by exposure to novel environments is intact in DANB-lesioned animals. This is consistent with the lack of effect in the social interaction test mentioned above.

A10.3.2.9 Conditioned inhibition, latent inhibition, blocking

Dorsal bundle lesions produce loss of Kamin's blocking effect, and improve non-reversal shift learning (Lorden *et al*. 1979; Mason and Fibiger 1979*d*), but do not affect latent inhibition (Tsaltas *et al*. 1984).

A10.3.2.10 Distraction experiments

The effects of distracting stimuli are mixed. Roberts *et al*. (1976) found that DANB lesions increased the distraction produced by flashing overhead lights and a change in floor covering in rats running for food in an alley; and Mason and Fibiger (1978*a*) observed the same with an overhead light in an operant chamber. However, with a light on the front panel of the operant chamber there was *reduced* distraction, and with tones there was no effect (Mason and Fibiger 1978*a*, 1979*c*; Owen 1979). By contrast, Crow *et al*. (1978) found reduced distraction of a licking response by tones. Not only is it difficult to see a consistent pattern in these results, but whatever pattern there may be does not appear to be equivalent to the equally patchy pattern of results with septal and hippocampal lesions. It seems likely that distraction per se is not affected, but that dorsal bundle lesions interact with other features of behaviour elicited or suppressed by the experimental situations.

A10.3.2.11 Counterconditioning and toughening up

DANB lesions eliminate the partial reinforcement extinction effect (PREE) with a short intertrial interval and up to 50 acquisition trials (Owen *et al*. 1977, 1982; Owen 1979), and impair on-the-baseline counterconditioning with a random interval (RI) 64-s schedule (Tsaltas *et al.* 1987). However, surprisingly, if a 24-hour intertrial interval is used (which demonstrates the most robust effects of anxiolytic drugs), then there is no effect on the PREE and, indeed, no effect on resistance to extinction. Less surprisingly, given the effects of septal lesions (see Appendix 8), DANB lesions do not effect the PREE if 100 acquisition trials are given. These results are considered further in Appendix 9.

A10.3.3 Locus coeruleus cell firing

Activity in the locus coeruleus can be recorded directly or can be inferred from, for example, the turnover of noradrenaline. There are relatively few studies using direct recording and so we will first consider the results of more indirect methods.

Stone (1975, Table IV) reviewed a large number of studies in which the turnover of noradrenaline in the brain (thought to reflect the rate of impulse transmission in noradrenergic neurons) was observed to rise after footshock, cold or heat stress, immobilization, forced exercise, handling, or various other stressful procedures. The same effect is produced by exposure to a CS for shock without the shock itself (Tilson *et al.* 1975), matching the release of corticosterone by such a stimulus (Brady 1975*a*,*b*); but a predictable shock produces less effect than an unpredictable one (Tsuda *et al*. 1989). Stress has also been reported (De Pottier *et al*. 1976) to increase the release into cerebrospinal fluid of dopamine beta-hydroxylase, the enzyme which catalyses the formation of noradrenaline from dopamine. The link with stress is made stronger by the fact that the locus coeruleus is one of only a modest number of brain stem sites in which the neurons contain, and presumably release, corticotropin-releasing factor (Austin *et al.* 1995). (The fact that the cholinergic pedunculopontine tegmental nucleus is one of the other sites is something to which we return in the section on cholinergic systems.)

The general increase in noradrenergic activity seen in the whole brain under conditions of stress has also been demonstrated to occur specifically in the DANB. Thus, Corrodi *et al*. (1971) and Lidbrink *et al*. (1972) showed that immobilization or

shock increases noradrenaline turnover in forebrain tissue dissected out from the rest of the brain. This appears to be a direct effect of corticotropin-releasing factor on the brain rather than an effect mediated by the pituitary–adrenal axis (Smagin *et al*. 1995; see also Valentino *et al*. 1993).

A critical observation for our theory is that the stress-induced increase in turnover of noradrenaline is prevented by the administration of barbiturates, benzodiazepines, alcohol, or meprobamate (Corrodi *et al*. 1971; Lidbrink *et al*. 1972, 1973; Taylor and Laverty 1973), and barbiturates also reduce stress-induced noradrenaline release (Ida *et al*. 1990). In agreement with these observations, Segal (1978) has reported that aversive stimuli (e.g. a pinch of the tail or leg) increase firing rates in locus coeruleus neurons; while Pohorecky and Brick (1977) report that alcohol decreases firing rates in the locus coeruleus. By contrast, buspirone produces, if anything, an increase in locus coeruleus firing (Trulson and Henderson 1984; Wilkinson *et al*. 1987). However, since the effects of buspirone on septal driving of hippocampal theta rhythm are the same as those of dorsal bundle lesions (Chapter 9), it seems likely that this increase in firing of locus coeruleus cells is an indirect result of presynaptic *blockade* of release of noradrenaline from nerve terminals in areas such as the hippocampus. Thus, the effects of all anxiolytics would be to reduce the release of noradrenaline in the septo-hippocampal system.

Stress-induced increase in noradrenergic activity can be detected in the hippocampus itself. Thus, the activity of tyrosine hydroxylase (the rate-limiting enzyme for the synthesis of noradrenaline) is elevated in synaptosomes (pinched-off nerve terminals) prepared from hippocampi dissected from the brains of rats shocked or handled shortly before death (Fillenz *et al.* 1979). This change in tyrosine hydroxylase activity may indicate (Boarder and Fillenz 1978, 1979) an increased rate of impulse traffic in hippocampal noradrenergic terminals.

However, Boarder *et al*. (1979) found an increase in the same tyrosine hydroxylase activity in rats trained to run for reward on a CRF schedule in a runway, compared to handled but untrained controls. This result raises the possibility that the DANB carries signals of reward. This view is also supported by the fact that locus coeruleus stimulation and CSs for food exert similar facilitatory effects on transmission round the hippocampal circuit (Segal 1977*a*,*b*), and that stimulation of the locus coeruleus in human subjects appears to produce relaxation and perhaps an increased clarity of thought with no sign of anxiety (Libet and Gleason 1994).

Boarder *et al*. (1979) also tested a group of rats trained with partial reinforcement (PRF). The PRF-trained animals displayed levels of tyrosine hydroxylase activity which were significantly lower than those seen in the CRF group, and which did not differ from those in the handled controls. This suggests that the non-rewarded trials in the PRF schedule actively returned levels of tyrosine hydroxylase activity to the baseline from which rewarded trials had raised them.

Thus, release of noradrenaline cannot provide a simple reward signal (since the signal is low while animals maintain good performance on the PRF schedule); nor, surprisingly, does it indicate omission of reward. The latter function might have been expected, given that stressful events activate the DANB and that corticosterone is released in response to reward omission (Coover *et al.* 1971) as well as other

stressors. Furthermore, injection of GABA antagonists into the locus coeruleus produces a strong behavioural activation, including elements of escape behaviour (but also including seizure-like activity; Priolo *et al*. 1991).

As with the serotonin system, then, we must look to some more general function to account for these anomalies. Activation of the DANB by novel stimuli, punishment, and reward suggests a view of the DANB close to the old notion of the ascending reticular activating system (Magoun 1963; Gray 1964; Segal 1980). This view has the advantage of assuming no specificity or detail in the information carried by the small number of cells in the locus coeruleus; and it is consistent with the fact that the DANB plays a central role in the functions of the ascending reticular activating system (Hobson and Brazier 1980), although the nature of this role remains obscure.

It was proposed by Jouvet (1969, 1972), on the basis of pharmacological and lesion evidence, that the locus coeruleus is responsible for REM (or paradoxical) sleep. This hypothesis seems consistent with the role of the DANB in the theta rhythm (Appendix 5) and the strong presence of theta during REM sleep (e.g. Winson 1990). However, the DANB does not seem to be essential for normal REM sleep, although it may play a modulatory role (Jacobs and Jones 1978). It is even possible that the locus coeruleus *inhibits* REM sleep (McCarley 1980). Certainly, DANB activity (like raphe activity, see above; but unlike cholinergic activity, see below) is lowest in REM sleep, intermediate in non-REM sleep, and greatest in waking (McCarley 1980; Segal 1980). Also, noradrenergic input depresses sleep-active neurons and excites waking-active ones (Osaka and Matsamura 1994). This pattern makes it tempting to equate activity in the DANB with a general arousal function. However, while cells in the locus coeruleus are normally reported to be silent during REM sleep, nonetheless dorsal bundle lesions eliminate the *augmentation* of REM sleep which occurs following learning (Bloch *et al.* 1978; Leconte and Hennevin 1981). Since dorsal bundle lesions do not block either sleep generally or REM sleep in particular, it seems likely that modest activity in the locus coeruleus contributes to REM sleep only following learning, accounting for the fact that such activity is not observed under normal recording conditions.

The general lack of effect of dorsal bundle lesions on major components of the sleep–waking cycle is consistent with the fact that, in the transition from REM sleep to waking, locus coeruleus 'neurons return to waking activity either coincident with or slightly after the cessation of paradoxical sleep' (Aston-Jones *et al*. 1991, p. 504). This observation suggests that, rather than directly controlling sleep–waking transitions, locus coeruleus activity relates to some function required predominantly during waking.

In the remainder of this section, we consider the possibility, suggested by Aston-Jones and his co-workers (e.g. Aston-Jones *et al.* 1991), that the locus coeruleus is a critical component of systems which regulate attentional state, or vigilance. A function of this kind is consistent with the lack of effect of dorsal bundle lesions on the sleep–waking cycle; and, if formulated suitably, could account for an increase in locus coeruleus activity during paradoxical sleep following learning. (This increase remains to be demonstrated directly, but is a reasonable inference from the results of Bloch *et al.* 1978 and Leconte and Hennevin 1981, described above.) The hypothesis proposed by Aston-Jones *et al*. might also account for the results of Boarder *et al*. with partial

reinforcement, discussed above. It is known that partial reinforcement produces a broadening of attention, that is it increases the number of stimulus dimensions about which an animal will learn (McGonigle *et al*. 1967). The locus coeruleus is active during continuously rewarded training. On an attentional/vigilance hypothesis, this would increase attention to a limited number of stimuli associated with reward. To achieve the broadening of attention required when reward is unreliable, the activity in the locus coeruleus would have to be reduced.

The notion that the locus coeruleus is involved in the focusing of the animal's attention on some limited set of stimuli is consistent with the fact that, unlike the raphe (see above), this nucleus is highly activated by unconditioned stimuli which elicit orienting responses and 'by stimuli which are not themselves intense or conspicuous, but are salient to the animal by virtue of conditioning' (Aston-Jones *et al*. 1994, p. 4468). Particularly compelling are the data on locus coeruleus activity during performance of an 'oddball' task, in which rare target stimuli are presented in the context of non-target distractors. The locus coeruleus produces phasic excitatory responses to the target stimulus, but to none of the other stimuli (including reward) occurring during the task. When the significance of the stimuli is changed, coerulear responses shift to the new target stimulus (Aston-Jones *et al*. 1994; see also Rajkowski *et al*. 1994).

A10.3.4 Functions of the DANB

Before considering the possible functions of the DANB, we should briefly preview the relationship between the behavioural effects of lesions to the DANB and those of lesions to the amygdala and septo-hippocampal system. What is particularly striking is how few cases there are where an effect of DANB lesions is not matched by the effects of septo-hippocampal lesions (as opposed to vice versa). Given the extensive influence of the DANB on the whole cortical mantle, as well as subcortical areas such as the amygdala, this is surprising. A possible explanation is suggested by the many cases in which an effect of DANB lesions is particularly evident only if there is additional damage to the pituitary–adrenal axis (or where the lesion interacts with other aminergic systems; see below). It thus seems possible that the DANB provides a subtle influence that becomes obvious with respect to many of its targets only when there is disturbance of additional systems which normally provide a degree of redundancy.

On this view, the DANB input to the hippocampus would be characterized by lesser (although perhaps not nil) redundancy than its input to other structures, with consequences evident in the case of only some elements of the septo-hippocampal syndrome (resistance to extinction, rearing, spontaneous alternation, etc.). With regard to these elements, DANB lesions produce substantial effects, effects moreover that are almost identical to those of anxiolytic drugs and septal and hippocampal lesions. Possibly again because of redundancy, there are also a number of elements in the septo-hippocampal syndrome on which DANB lesions produce no effect. In this way, these lesions dissect the septo-hippocampal syndrome into dissociable components. Thus, take reversal learning, open-field ambulation, intermittently rewarded bar-pressing, Sidman avoidance, performance on DRL schedules, and possibly learning in the Morris water maze—performance on all of these is present in the septo-hippocampal syndrome but not the DANB one. The pattern of positive results

suggests that the function of the DANB is particularly important for the septo-hippocampal system but less critical for the operation of its other targets. The pattern of negative results suggests that DANB input is necessary for only some but not all septo-hippocampal functions.

There is, however, an alternative possible account of this pattern of partial overlap between the effects of DANB lesions and the anxiolytic/septo-hippocampal syndrome. DANB lesions reproduce the effects of anxiolytic drugs on septal driving of theta, but not on the frequency of reticularly elicited theta (Appendix 5). Lesions of this pathway may therefore select out the 'septal' as opposed to 'reticular' component of the parent syndrome. The pattern of results with DANB lesions may therefore provide clues to both the functions of the DANB generally and the nature of two dissociable sets of functions subserved by the septo-hippocampal system. It is, however, less easy to determine precisely what the function of the DANB might be.

We have considered the effects on hippocampal electrophysiology of the noradrenergic input in more detail in Appendix 5 and so provide only a brief summary here. Like input from the raphe nuclei, input from the DANB increases the signal-to-noise ratio in the hippocampus, facilitating transmission round the trisynaptic circuit. The DANB input also facilitates or enables long-term potentiation; and it increases interaction between the two hippocampi. It does not (as noted above) influence the frequency of the theta rhythm, which is controlled by the septum and produced by both the hippocampus and entorhinal cortex. An inhibitory action on the locus coeruleus can duplicate, therefore, some but not all of the neurophysiological effects of anxiolytic drugs on the septo-hippocampal system. Inhibition of the DANB is likely to be more pro- than anticonvulsant (Ferraro *et al*. 1994; Kokaia *et al*. 1994), but this does not necessarily set it apart from anxiolytics, since buspirone is not anticonvulsant.

As previously concluded (Appendix 5), long-term potentiation of the perforant path–dentate synapses may represent a signal corresponding to the command 'familiar–ignore', since it is accompanied by a reduction in responses elicited by natural stimuli in the remainder of the hippocampus. In this context, additional noradrenergic input, which would simultaneously augment long-term potentiation in these synapses *and* facilitate impulse traffic in the rest of the hippocampus, might correspond to 'familiar–but do not ignore', as required for example in the case of familiar stimuli associated with biological reinforcers: equating, perhaps, to the command 'important–check carefully'. A function of this kind is likely to be of particular value under conditions of conflict, that is, in states of anxiety. This hypothesis is consistent with a number of facts already reviewed: that stress increases impulse traffic in the DANB; that the anxiolytic drugs impair conduction in the DANB; that this impairment is seen especially under conditions of stress; and that this impairment, or an equivalent net effect, is produced by all classes of anxiolytic drugs. It is, in addition, not inconsistent with the idea, also discussed above, that the DANB signal corresponds to a fairly general command: 'be vigilant'. This general function would also be operative when environmental stimuli are novel. Under these circumstances there would be no long-term potentiation of the perforant path–dentate synapses, but intra-hippocampal noradrenaline release would still boost transmission round the trisynaptic circuit.

The major null features of the DANB syndrome are the following: there is no change in responses to reward or shock, or in the learning of simple rewarded responses, one-way active avoidance or Sidman avoidance; there is no change in general activity in a novel environment, distractibility, habituation, fearfulness, conditioned taste aversion; there is no change in the frustration effect, the Crespi depression effect, off-the-baseline conditioned suppression, latent inhibition, reversal learning, and DRL; and, at least under most conditions, no effect in the Morris water maze.

The major positive features of the DANB syndrome are: a clear reduction in resistance to extinction under most but not all conditions; impaired successive discrimination, Kamin blocking, and to a lesser extent on-the-baseline conditioned suppression; reductions in rearing, spontaneous alternation, response to stimulus change; some impairments in spatial learning; and elimination of the partial reinforcement acquisition effect and of the partial reinforcement extinction effect, but only in limited circumstances (see Appendix 9).

Recall that (as reviewed above) activation of the DANB appears to occur under conditions of stress, that is under approximately the same conditions which would cause the release of corticosterone. In particular, painful stimuli activate the locus coeruleus (Segal 1978), and release of the locus coeruleus from GABAergic inhibition produces behaviour in animals suggestive of panic attacks (Priolo *et al*. 1991). However, reward also appears to activate the locus coeruleus, an effect which is removed by training under a partial reinforcement schedule.

What hypotheses can account for all the above results? It is easier, in fact, to see which hypotheses fail. The DANB cannot be primarily concerned with positive reinforcement, since simple rewarded behaviour is normal in lesioned rats (see also first edition, pp. 313–16). A general role in learning or consolidation is also ruled out by a variety of unaffected tasks. A restricted role in consolidation of some tasks is possible (Crow and Wendlandt 1976; Zornetzer and Gold 1976; Kovacs *et al*. 1979; Leconte and Hennevin 1981), perhaps via an interaction with REM sleep (see also Winson 1990). However, considerable further data on both the effects of DANB lesions and the role of REM in consolidation are needed before any specific suggestion can be made about this possibility. A general role in anxiety is similarly ruled out, although inhibition of the DANB could underlie *part* of the behavioural profile of the anxiolytic drugs.

One suggestion made by Segal and Bloom (1976) on the basis of their electrophysiological experiments (Appendix 5), and by Mason and Iversen (1977, 1979) on the basis of the behavioural effects of DANB lesions, is that the DANB plays a role in inhibiting attention to irrelevant stimuli. This is essentially the same as Douglas's (1967) theory of hippocampal function. Unfortunately, as is often the case when the concept of attention is introduced in a physiological context, the application of this hypothesis to the data has largely been ad hoc and lacking in precision. The theory might seem strong because it has grown out of the data on DANB lesions. But this closeness to the data turns out to be more of a weakness than a strength. It is usually impossible to tell in advance what is a 'relevant' and what an 'irrelevant' stimulus, and so the theory lacks predictive power. Furthermore, 'irrelevance' would appear to be a highly processed construct, fitting rather ill with the tiny size of the locus coeruleus.

As an example of this imprecision, consider Owen's (1979; McNaughton *et al*. 1984) finding that DANB lesions eliminate the response to stimulus change. In this experiment the animal is placed in the stem of a T-maze of which one arm is white and one is black. It is left there, separated from the arms by transparent partitions, for 3 min. It is then taken out, the arms are changed so that both are now black or both are white, the partitions are removed, and the rat is returned to the maze. Normal rats choose the changed arm about 75 per cent of the time; rats with DANB lesions—like those drugged with sodium amylobarbitone (Ison *et al*. 1966; Owen 1979)—chose at random.

Can the results be predicted from Mason and Iversen's (1979) hypothesis? Evidently, the answer to this question depends on whether the brightness of the arms of the T-maze is relevant or irrelevant (to what?). If it is 'relevant', the lesioned animal might be expected to respond less to the change in brightness than controls (because it is busy responding to other 'irrelevant stimuli'). If it is 'irrelevant', the lesioned animal would presumably respond more than controls to a change in brightness. Thus the experiment can be explained post hoc, but its results cannot be predicted in advance.

A further example of the dangerous flexibility of the 'attentional irrelevance' hypothesis comes from a paper by Mason and Fibiger (1978*a*). When they find that DANB-lesioned animals are more distracted by an overhead light, less distracted by a light in front of the eyes, and no different from controls when the distractor is auditory, they include all the results within their theory by the simple expedient of postulating the appropriate saliences for the different types of stimuli.

Mason and Iversen (1979) have attempted to increase the power of their approach by wedding it to the Sutherland and Mackintosh (1971) general theory of selective attention. But the one case to which they have so far fully applied this analysis fails to square with their predictions. This is the blockade of the PREE by dorsal bundle lesions (Owen *et al*. 1977, 1981).

Mason and Iversen (1979) argue that the increased resistance to extinction produced by dorsal bundle lesions in CRF-trained animals arises because the lesioned animals attend to more stimuli; and this, following the predictions made by Sutherland and Mackintosh (1971), would result in the equivalent of a PREE. If this were true, the dorsal bundle CRF, dorsal bundle PRF, and control PRF groups should all show the same resistance to extinction, and this should be greater than for control CRF animals. However, like anxiolytic drugs, dorsal bundle lesions reduce resistance to extinction in PRF animals as well as increasing it in CRF animals, thereby abolishing the PREE (Owen *et al*. 1977, 1981). Furthermore, while Sutherland and Macintosh's theory provides a good account of the shifts in attention during partially rewarded acquisition, it turns out that these shifts themselves cannot account for increased resistance to extinction (McFarland and McGonigle 1967).

In an important modification of our own position on this subject, we have concluded (see Appendix 9, Section 9.8) that simple extinction and the partial reinforcement effect depend on independent processes. On our new analysis, the former depends straightforwardly on behavioural inhibition. However, the partial reinforcement extinction effect does not result from cancellation of that behavioural inhibition. Rather, it depends on latent inhibition of responses to frustrative stimuli (Appendix

9.8). This conclusion is at variance with Mason and Iversen's account, particularly in respect of their proposed link to Sutherland and Mackintosh's theory. In addition, the new analysis opens up some novel possibilities for the role of the DANB.

Aston-Jones' view of this role (see above) is that output from the locus coeruleus focuses attention; or, conversely, a lack of such output results in broader stimulus processing. Broad stimulus processing of this kind is exactly what occurs in normal animals trained on a partial reinforcement schedule. Aston-Jones' hypothesis is not ad hoc, since 'relevance' does not enter into it. As noted above, the hypothesis deals successfully with the fact that tyrosine hydroxylase activity in the hippocampus (and, by inference, locus coeruleus activity) is similar in partially reinforced and untrained animals, but different in continuously reinforced animals (Boarder *et al*. 1979). It can also, with some additional assumptions, accommodate the overall effects of DANB lesions on resistance to extinction better than does the rival view proposed by Mason and Iversen.

We suppose that resistance to extinction is, at least in part, due to the focusing of attention during extinction testing on stimuli associated with non-reward. The greater this attention, the more rapid is the inhibition of responding that leads to the non-reward-associated stimuli. In the absence of the DANB, focused attention to these stimuli should be reduced, and so also the capacity to inhibit the responses that lead to non-reward—that is, resistance to extinction should be increased, as in fact observed. (This part of the argument is similar to Mason and Iversen's 1979 treatment of the increased resistance to extinction seen in DANB-lesioned animals after CRF training.) In line with the general body of evidence implicating the septo-hippocampal system in simple extinction (see Appendices 8 and 9), as well as the measurements of hippocampal tyrosine hydroxylase activity reported by Boarder *et al*. (1979; see above), we may further suppose that this influence of the DANB is mediated via its terminals in the hippocampus.

The loss of the PREE after DANB lesions would then be accounted for in a similar way, but with one variation. As for simple extinction, we assume that the loss of noradrenaline release after DANB lesions decreases the salience of non-reward. But the consequences of this change now take effect also during acquisition. As originally suggested by Joram Feldon (personal communication, 1985; see Gray *et al*. 1991), we have interpreted the PREE as reflecting latent inhibition of stimuli associated with non-reward (Appendix 9.8). Latent inhibition is a positive function of stimulus intensity (Lubow 1959; Schmajuk *et al*. 1996, Fig. 10). Thus, a reduction in the salience of non-reward-associated cues, consequent upon a DANB lesion, should reduce latent inhibition of these cues and so the PREE. However, this effect must be mediated by DANB terminals in a region other than the hippocampus, for reasons considered in Appendix 9.8. The most likely alternative is the entorhinal cortex. In line with the arguments adduced above, this effect of the DANB innervation of the entorhinal cortex would be confined to stimuli important for survival. Thus, DANB lesions would not be expected to give rise to a general loss of latent inhibition to neutral stimuli. In this way, it is possible to account both for the positive effects of DANB lesions on the PREE and for the absence of any effect on latent inhibition as such.

Before following this argument too far, recall that the information carried by a pathway need not be used under all circumstances by its target structures. Thus, there is evidence that the DANB is activated by information about reward, punishment, and novelty. On the other hand, evidence from lesion experiments shows that the dorsal bundle does not subserve positive reinforcement itself, although (as just indicated) it may subserve behaviour related to non-reward. We can reconcile these data on the basis of three linked assumptions.

First, we assume that the locus coeruleus is activated by all events of potential importance to the animal's survival: it is a general alerting or alarm system (see also Redmond 1979). Indeed, the locus coeruleus has been viewed as the CNS equivalent of the sympathetic nervous system (e.g. Aston-Jones *et al.* 1991; Van Bockstaele and Aston-Jones 1995). This is essentially the same as the older view of the ascending reticular activating system as a general arousal mechanism (Magoun 1963; Gray 1964). However, we must refine this concept somewhat, in that locus coeruleus (LC) cells 'decreased tonic discharge . . . during certain high arousal behaviours (grooming and consumption) when attention (vigilance) was low. . . . The most effective and reliable stimuli for eliciting LC responses were those that disrupted behaviour and evoked orienting responses' (Aston-Jones *et al.* 1991, p. 501). These observations are consistent with the view that 'LC cells respond to novelty or change in incoming information, but do not have a sustained response to stimuli, even when these have a high level of biological significance' (Sara *et al.* 1994). On this view, output from the locus coeruleus increases vigilance, consistent with electrophysiological data showing that locus coeruleus input increases the signal-to-noise ratio (see below). Increased signal-to-noise ratio will affect stimulus processing in target structures of the DANB, including the septo-hippocampal system, amygdala, and nucleus accumbens (see Appendix 9). This perspective is akin to Mason and Iversen's (1979) view that the DANB is required for attention to relevant stimuli. It differs, however, in that: (1) the DANB is proposed to boost attention to some stimuli more than others; (2) 'relevance' of stimuli is not a factor as such; and (3) the effect is presumed to be restricted to, for example, hippocampal processing rather than being totally general. The predictions of this hypothesis depend, therefore, not only on the information postulated to be available in the dorsal bundle, but also on the functions of the specific target structures; and, in particular, on whether those targets are themselves involved in the control of behaviour. We detail below the postulated effects of noradrenaline release on these structures.

Second, we suppose that the 'arousing' effect of the dorsal bundle output extends to motor mechanisms as well as to stimulus processing (see also Aston-Jones *et al*. 1991, p. 514). Whatever the animal does, it does more vigorously if the dorsal bundle is active (Gray 1964, 1975; Gray and Smith 1969). Thus, activity in the DANB contributes to the 'increment arousal' output of the behavioural inhibition system (Chapter 3). This aspect of dorsal bundle output is exemplified by its role in the partial reinforcement acquisition effect. However, consistent with a role as part of the behavioural inhibition system, the DANB is not involved in all aspects of arousal; for example, lesions of this pathway do not affect the frustration effect or active avoidance behaviour.

Third, we suppose that the dorsal bundle plays only a limited role in behavioural inhibition as such. In particular, we see it as involved largely in those aspects of

behavioural inhibition which are based on the resolution of conflict between *stimulus* alternatives, not in those based on the resolution of conflict between *response* alternatives. This conclusion is based on the lack of involvement of the DANB in tasks such as DRL, its involvement in successive discrimination, and its modest effects in apparatus such as the water maze. Thus, of the three outputs of the behavioural inhibition system, the dorsal bundle is somewhat more concerned with increased arousal and increased attention, and somewhat less so with behavioural inhibition. As we noted, its role in determining resistance to extinction can be accounted for by the capacity to focus on environmental stimuli associated with consistent non-reward. Its lack of influence on performance on DRL schedules is perhaps attributable to the inconsistent nature of the rewards received (which would render the locus coeruleus inactive).

Fourth, we suppose that the outputs of the behavioural inhibition system that are not modulated by the DANB, particularly in response to stimuli associated with punishment, are supplied mainly by the raphe serotonin system. As noted in the first part of this appendix, we see the latter as being involved in a form of 'motor attention'. We elaborate on this aspect of our theory later.

As outlined by McNaughton and Mason (1980) and Segal (1980) the underlying neurophysiological mechanism by which the noradrenergic neurons operate is as follows. As shown by a number of investigators, stimulation of the locus coeruleus, or direct application of noradrenaline to a target organ served by the dorsal bundle, has two effects: the spontaneous firing rate of neurons in the projection area of the locus coeruleus is reduced, but their response to other afferents is increased (Foote *et al*. 1975; Siggins and Hendriksen 1975; Freedman *et al*. 1976; Moises *et al*. 1978; Waterhouse *et al*. 1978; Woodward and Waterhouse 1978); in consequence, the signal-to-noise ratio with respect to the non-noradrenergic afferent is increased. This phenomenon has been observed within the hippocampal formation and, in particular, in response to stimulation of the perforant path (Segal and Bloom 1976; Assaf 1978; Assaf *et al.* 1979; see Appendix 5). Such an increase in signal-to-noise ratio would increase contrast in, for example, the current pattern of entorhinal inputs to the hippocampal formation.

There is one apparent problem in treating the DANB input to the hippocampus as adding an 'important' or 'be vigilant' label to other afferent inputs. Neither single-unit experiments nor investigations of evoked potentials suggest that the hippocampus differentiates between stimuli associated with appetitive and aversive events respectively; both kinds of association seem to be equally effective in facilitating transmission round the hippocampal circuit. Yet the experiments on hippocampal lesions reviewed in Appendix 8 suggest that reward and punishment are dealt with quite differently. This apparent problem disappears if we remember that active avoidance learning is unimpaired by hippocampal lesions, just as is active reward learning. Thus, the DANB sends an 'important' label to the hippocampus which, under normal active learning conditions, has no obvious functional effect since no inhibitory output is required. Eventually, as learning proceeds, a model of the reinforcers is created by the neocortex. This then provides a second input to the hippocampus which, because of the concurrent input from the DANB, undergoes LTP in the dentate.[1] In simple active learning, LTP would not occur in later stages of the hippocampal circuit because of the lack of conflict. Note that this explanation

accounts for the fact that resistance to extinction is seen after DANB lesions in aversive as well as appetitive tasks.

It remains to deal with the role of the DANB in relation to novelty. The locus coeruleus does not appear to have the information processing capacity to discriminate between novel and familiar (or relevant and irrelevant) stimuli. Nor do many of the known afferents to the locus coeruleus look likely as the origin of this type of information. Nonetheless, as we have noted, there is evidence that locus coeruleus neurons increase their firing rate in response to novel stimuli (Foote *et al.* 1978; Jones *et al.* 1978). To resolve this conundrum, we assume that any sufficiently salient stimulus initially activates the locus coeruleus. We next assume that a model of the stimuli, once it is completed elsewhere in the brain, prevents activation of the locus coeruleus by inhibiting whatever area provides the relevant input. Aston-Jones *et al.* (1991, pp. 515–16) suggest that the immediate source of the relevant information is the nucleus prepositus hypoglossi, which they postulate 'may be concerned with the initiation and coordination of wholistic orientation responses rather than just the ocular components' with which it has classically been associated.

Particularly given the possibility that, for many structures, locus coeruleus input is one of a number of redundant alternatives, it will be better to leave its global functions for assessment by future experimentation. With respect to the septo-hippocampal system, however, we have argued that there is less redundancy. For this system, at least, it appears that we can treat input from the locus coeruleus as providing a non-specific signal encompassing reward, punishment, and (highly salient) novelty. Possibly this signal equates with the message: 'important' or 'be vigilant'. But even this conclusion needs to be treated with caution. We have already discussed the possible role of a change in the signal-to-noise ratio with respect to serotonergic input to the septo-hippocampal system; we have now put forward a similar idea with respect to the noradrenergic input; and we are about to find equivalent data in relation to cholinergic input. Accordingly, we reconsider the possible differences between these inputs at the end of the appendix.

A10.3.5 Other approaches to noradrenergic function

The above analysis has concentrated on the results obtained with highly specific neurotoxic lesions of the dorsal ascending noradrenergic bundle. But, before we leave the topic of noradrenaline, there remain important data from experiments in which central noradrenergic function has been manipulated pharmacologically.

Stein's group (Wise *et al.* 1973) reported a series of experiments in which noradrenaline and a variety of other substances were injected into the cerebral ventricles of rats while they performed various rewarded and/or punished tasks in an operant chamber. Intraventricular noradrenaline did not increase the suppression of punished bar-pressing, but rather decreased it. This finding does not appear consistent with the effects of dorsal bundle lesions reviewed so far, and so some account must be found for it. There are two possible such accounts.

The first is that intraventricular noradrenaline acts on structures other than the septo-hippocampal system. Given the putative redundancy discussed earlier, we suppose that dorsal bundle lesions would have relatively little effect on these structures. The

bulk of the noradrenergic innervation of the hippocampal formation is located in the hilus of the dentate gyrus, some way from the ventricles. It is possible that other dorsal bundle terminals are more easily reached by the intraventricular route and that these play a role in mediating behavioural responses to reward, as postulated by Stein (1968). One possible site is the amygdala. Consistent with this possibility, Margules (1968, 1971) showed that noradrenaline injected directly into the amygdala alleviated punished suppression of bar-pressing. This raises the possibility that noradrenergic input to the hippocampus and amygdala may play a 'switch over' role similar to that of serotonergic input to the amygdala and dorsal periaqueductal grey. Noradrenergic input may increase the contribution of the hippocampus to behavioural inhibition by increasing the net negative valence of stimuli, while decreasing the contribution of the amygdala by increasing net positive valence.

The second possible account of the effects of intraventricular noradrenaline is that they are due to an action on presynaptic receptors. Wise *et al*.'s (1973) experiments showed that noradrenaline produced its effects via an alpha- not a beta-noradrenergic receptor. Alpha-noradrenoceptors in the brain have often been identified as presynaptic (Starke 1979). As with the 5-HT_{1A} autoreceptors in the serotonergic system, noradrenaline acting at such presynaptic sites can decrease release of noradrenaline elsewhere (Langer 1979). An effect of this kind would completely reverse Wise *et al*.'s (1973) interpretation of their findings. If this account is correct, dorsal bundle lesion should abolish the effects observed by Wise *et al*. (1973) with intraventricular noradrenaline.[2] This experiment has still not, to our knowledge, been performed.

It should be noted that Wise *et al*.'s (1973) pharmacological analysis also makes it unlikely that the effects they observed were due to an action on the hippocampus. The postsynaptic receptor in this structure is of the beta variety (Segal and Bloom 1974; Atlas and Segal 1977), as it apparently is also in the cingulate cortex (Melamed *et al.* 1977; Dillier *et al*. 1978).

The anti-anxiety drugs are not alone in their affinity for the noradrenergic system originating in the locus coeruleus. Opiates, such as heroin and morphine, also exert a powerful effect on coerulear neurons.

Endogenous opiate receptors (Kuhar *et al*. 1973; Hughes *et al*. 1975) are particularly rich in the locus coeruleus (Pert and Snyder 1973; Pert *et al*. 1975). Met-enkephalin has been demonstrated in axo-dendritic terminals (Pickel *et al*. 1979) and iontophoretically applied opiates depress the firing of coerulear neurons (Bird and Kuhar 1977; Guyenet and Aghajanian 1977; Young *et al.* 1977). This is the basis for an interesting account for some opiate withdrawal symptoms proposed by Gold *et al.* (1978). According to these authors, exogenous opiates reduce activity in the locus coeruleus. Thus, when the opiate is withdrawn from an addicted individual, there ensues a rebound hyperactivity in coerulear neurons, or more likely supersensitivity in the receptors that are postsynaptic to terminals from these neurons; this hyperactivity then gives rise to the withdrawal syndrome.

In support of this hypothesis it has been demonstrated that clonidine, a drug which inhibits the firing of neurons in the locus coeruleus (Cedarbaum and Aghajanian 1976; Tang *et al*. 1979), eliminates the symptoms of opiate withdrawal in man (Gold

et al. 1978, 1979*a*). Clonidine is an alpha-adrenoceptor agonist which appears to act, among other sites, on coerulear autoreceptors (Aghajanian *et al.* 1977). Thus, clonidine acts by a different molecular mechanism than the opiates, but, in the locus coeruleus, produces the same final effect: reduced activity. (If, however, alpha-2 receptors are blocked, clonidine can be shown to have a residual excitatory effect on coerulear neurons via excitatory amino acid receptors; Ruiz-Ortega *et al*. 1995.)

If opiate withdrawal symptoms are due to hyperactivity in the locus coeruleus, and if (as proposed here) activity in this system contributes to anxiety, it follows that the opiate withdrawal syndrome is a form of anxiety, perhaps a particularly intense form. This inference is supported by consideration of the actual symptoms that make up the syndrome. These include 'anxiety, yawning, perspiration, lacrimation, goose flesh, tremors, hot and cold flashes, increased blood pressure, insomnia, increased respiratory rate and depth, increased pulse rate, and restlessness' (Gold *et al.* 1979*b*). There are also symptoms less easily associated with anxiety, e.g. aching bones and muscles, nausea, and vomiting.

If this opiate withdrawal syndrome is equivalent to intense anxiety, and if it is, at least in part, due to hyperactivity in coerulear neurons or supersensitivity in the receptors on which they act, this may provide a clue as to the source of the autonomic symptoms of anxiety. It will be recalled that open-field defecation, which has been an excellent index of fearfulness in genetic experiments (Broadhurst 1960; Gray 1971; and see Chapter 12 in the printed text), is unaffected by either hippocampal or dorsal bundle lesions. The opiate withdrawal syndrome, however, is rich in autonomic symptoms, and these were all suppressed by clonidine (Gold *et al.* 1978, 1979*a*). It is possible, therefore, that the descending projections from the locus coeruleus (which are left intact by dorsal bundle lesions) are responsible. These projections could act in conjunction with the descending projections from the septal area to the hypothalamus (since septal lesions do reduce autonomic signs of fearfulness: Appendix 8); and in conjunction also with the descending projections from the amygdala, which we consider in Chapter 6 of the printed text (see Section 6.3.7).

A further deduction from the arguments pursued above is that clonidine should act like an anti-anxiety drug. Davis *et al.* (1979) showed that, like benzodiazepines (see also Appendix 2), clonidine impaired fear-potentiated startle, without affecting the unconditioned startle response. Similarly, clonidine eliminates the minimum in the septal theta driving curve, which would be predicted directly from its autoreceptor action, and reduces the frequency of theta elicited by reticular stimulation (via an indirect action on 5-HT_{1A} receptors; Coop *et al.* 1992). It thus has all three of the properties which cause us in Appendix 5 to link other anxiolytic drugs with actions on the hippocampus and amygdala.

Consistent with clonidine's action, the alpha-adrenoceptor antagonists piperoxane and yohimbine potentiated fear-potentiated startle, but not unconditioned startle, while the beta-receptor antagonist propranolol acted like clonidine. These results, then, are highly systematic: increased activity in noradrenergic neurons (a hypothesized effect of the alpha-antagonists) increases fear-potentiated startle; decreased noradrenergic activity (whether produced by an alpha-agonist or a beta-antagonist) decreases potentiated startle. Propranolol also has anti-anxiety-like effects on responding maintained on a DRL schedule (Salmon *et al.* 1989).

These results in animals are consistent with reports that, in man, yohimbine (Holmberg and Gershon 1961) and piperoxane (Goldenberg *et al*. 1947; Soffer 1954) produce feelings of anxiety; while propranolol is sometimes used as an anti-anxiety agent (Redmond 1979). However, propranolol does not appear to reduce cognitive aspects of anxiety. Rather, it is particularly useful in treating performance anxiety (e.g. in musicians), as it reduces the peripheral and autonomic signs of anxiety, such as tremor, which interfere with performance. In this, it is quite unlike the benzodiazepines. Furthermore, neither in animal experiments nor in the clinical literature (Gottschalk *et al*. 1974; Tyrer 1976) has the possibility been ruled out that drugs like propranolol act by way of a purely peripheral mechanism. However, given the data considered above on the dorsal bundle, a contribution from central action remains a possibility.

A10.4 The ascending cholinergic systems

The two monoamine systems considered so far are each likely candidates for 'the neural basis of anxiety', and indeed each has been proposed as such. Each is acted on by both classical and novel anxiolytic drugs, which depress their function (although with novel anxiolytics and noradrenaline we suggested above that the effect is achieved at the terminals and produces a rebound increase in locus coeruleus firing). The simplest resolution of this situation, then, is to assume that *both* contribute to anxiety. Indeed, concurrent effects on both systems can account for much of the behavioural profile of the anxiolytic drugs.

In this context, the results we review in the present section on cholinergic systems are somewhat surprising. We will see that anticholinergic treatment and, where this has been tested, lesion of ascending cholinergic systems, have not only behavioural effects that are quite similar to those seen after change in the noradrenergic and serotonergic systems, but also a similar effect at the neural level: changing the signal-to-noise ratio in targets such as the hippocampus and amygdala. Yet, in man, the anticholinergics are amnestic rather than anxiolytic; and some procholinergic drugs may even be anxiolytic (see Brioni *et al*. 1994; Garvey *et al*. 1994).

To resolve this apparent problem of similar behavioural profiles resulting from damage to nominally 'anxiolytic' and nominally 'amnestic' systems, we make two assumptions. The first is that 'anxiolytic' and 'amnestic' action are very closely allied, but not identical. This is, of course, a central tenet of our theory. Consistent with this assumption, benzodiazepines are likely to achieve at least some of their behavioural effects by the blockade of activated efflux of acetylcholine onto cortical targets (Anglade *et al*. 1994; Sarter and Brun 1994). Anticholinergic drugs, by contrast, probably do not produce their amnestic effects via release of endogenous benzodiazepine ligands (Duka *et al*. 1992). The second assumption is that there are detailed points at which interference with cholinergic systems produces effects that are quite different from those of interference with monoaminergic systems. To anticipate, we will argue that the cholinergic input is concerned more with the 'memory' end and the monoamines with the 'affective' end of a spectrum of activities which are all dependent on essentially the same basic neural system. This differentiation will be the business of the present section.

A10.4.1 Anatomy of the ascending cholinergic systems

In considering the neuroanatomy, the first point to note is that modern methods based on choline acetylase (the synthetic enzyme for acetylcholine) have produced a noticeably different picture to that originally proposed by Lewis and Shute (1967). Second, in many cases the cholinergic projections are accompanied by parallel non-cholinergic (frequently GABAergic) projections. Finally, and at present tentatively, the clearest picture is probably obtained if we divide the cholinergic systems into three groups: (1) a diverging ascending cholinergic system arising in posterior areas; (2) the medial septal/diagonal band complex (MS/DBB) in the basal forebrain; and (3) the nucleus basalis, substantia innominata, and magnocellular preoptic areas (NBM/PO) also in the basal forebrain. Cutting across this division, however, the posterior system has both direct and indirect projections to the two basal forebrain systems so that, taken as a whole, the three present the superficial appearance of a continuum.

The posterior cholinergic system (see review by Steckler *et al*. 1994*a*) arises in the pedunculopontine tegmental nucleus (PPT) and the laterodorsal tegmental nucleus (LDT) and innervates a variety of thalamic nuclei, the lateral hypothalamus, the superior colliculus, the substantia nigra, the basal forebrain, septum, and frontal cortex. There are also cholinergic projections from the PPT to the amygdala, but predominantly more non-cholinergic ones. In at least some cases, the cholinergic projections are intermingled with parallel GABAergic projections (Ford *et al*. 1995).

In describing the two basal forebrain groups we will largely conflate the results of Woolf *et al.* (1984) with those of Gaykema *et al.* (1990; see also Wainer and Mesulam 1990).

Figure 6.7 in the printed text shows the cholinergic and non-cholinergic projections from the MS/DBB. These have a fairly simple organization, with the route taken by the projection depending on the distance to be travelled. Thus, as with the ascending noradrenergic and serotonergic pathways, the cholinergic pathway takes a ventral, septal, and supracallosal route. The more caudal and ventral components of MS/DBB (the horizontal limb of the diagonal band; HDB in the figure) project to the most ventral areas both rostrally (olfactory bulb) and caudally (pyriform, entorhinal, ventral hippocampal cortices). As we move progressively rostral and dorsal from the diagonal band to the medial septum (from HDB to VDB—the ventral limb of the diagonal band—to MSm to MSl in the figure), the fibres take first dorsal and then progressively posterior routes to first dorsal and then progressively posterior targets. Thus, if the MS/DBB is seen as a semicircle and its targets as a nearly complete circle (open in the region of the ventral hippocampal formation, i.e. at a line between HFv and LEA on the figure), then the projections are essentially topographic, with clockwise movement around the MS/DBB arc resulting in clockwise movement round the target arc. This non-discrete topography is reminiscent of that shown by the noradrenergic system (see McNaughton and Mason 1980, pp. 162–3).

This picture of projections is, in general, largely applicable to both non-cholinergic and specifically cholinergic cells of the relevant nuclei. However, there may be some differences in the proportions of cholinergic and non-cholinergic cells involved, with

the projection from the MS to the entorhinal cortex being predominantly non-cholinergic, in contrast to that from the VDB and HDB.

The projections of the NBM/PO are not so clearly organized, at least on present data. However, once it is separated from the MS/DBB, there are signs of some degree of organization, with pyriform cortex receiving major input from the magnocellular preoptic area and the perirhinal and insular cortices from the nucleus basalis; while all three of these areas and the amygdala receive major input also from the substantia innominata. As with the MS/DB's topographical organization, this pattern involves considerable overlap.

A final organizing principle for this area is that 'the proportion of the total number of basal forebrain neurons innervating the limbic telencephalon that demonstrated ChAT-like immunoreactivity displayed a tendency to increase from archicortical to paleocortical and mesocortical fields. . . . 62% of the cells projecting . . . to the hippocampal formation, . . . for the pyriform and cingulate cortices . . . 79 and 95%, respectively [and] only 11% [for] olfactory bulb' (Woolf *et al*. 1984; compare 42% hippocampus, 64% cingulate cortex, and 15% olfactory bulb in Senut *et al.* 1989).

A10.4.2 Acetylcholine and behaviour

Unfortunately there are only limited data on the effects of specific cholinergic lesions on behaviour. We will, therefore, have to assess the functions of the cholinergic system largely from the effects of systemic (usually antimuscarinic) drug treatment. These have been studied and reviewed extensively over the years (e.g. Bignami 1976; Aigner 1995), and a link between the cholinergic system and behavioural inhibition has long been recognized (Carlton 1969).

Briefly summarized, anticholinergic drugs are like anxiolytics in impairing rearing, spontaneous alternation, extinction, successive discrimination, differential reinforcement of low rates of response, passive avoidance, and spatial learning, and in improving two-way and non-spatial active avoidance. Their effects on spatial learning extend to birds and to ecologically reasonable tasks (Mineau *et al.* 1994*a*,*b*; Köhler *et al*. 1996). As with hippocampal lesions (Appendix 8), prior experience with the apparatus prevents the spatial learning deficit (Saucier *et al*. 1996). However, anticholinergics are unlike anxiolytics in that they impair acquisition of a running response in a straight alley and acquisition of simultaneous discrimination, and do not increase responding on intermittent reinforcement schedules of bar-pressing. Thus, their effects appear to overlap to a large extent with those of the anxiolytic drugs. However, they also affect at least some learning which is not characterized by behavioural inhibition, and fail to affect some which *is* so characterized. It is also relevant that acetylcholine is released during acquisition of a simple lever-press task (Orsetti *et al*. 1996).

Of particular interest in relation to the possible links between cholinergic systems and anxiolytic action, Rodgers *et al*. (1990, p. 575) state that: 'while there is little doubt that manipulation of cholinergic function can rather specifically alter offensive behaviour in a variety of species, evidence also supports a role for central muscarinic receptors in defensive responding. . . . Antimuscarinics have . . . been reported to inhibit shock-induced defensive fighting in rats and mice . . . Under more naturalistic

test conditions . . . scopolamine reduced fear reactions in laboratory rats confronted with a cat . . . reduced fear was indicated by consummatory behaviour in the presence of the cat, more approaches to the cat enclosure and less freezing.'

Rodgers *et al*. (1990) analysed this issue further using elements of the fear and anxiety batteries described in Chapter 2 of the printed text. Scopolamine did not alter 'avoidance, freezing, defensive threat or attack in wild *Rattus rattus* confronted by the experimenter and other threat-related stimuli . . . During cat exposure, however, scopolamine hydrobromide (but not methylscopolamine) increased the amount of time spent in the vicinity of the cat, increased scanning and rearing, and reduced grooming behaviour. Although reliable, the latter effects were not pronounced' (Rodgers *et al*. 1990, p. 575). Considering the overall pattern of responding, Rodgeres *et al*. (1990, p. 581) conclude that scopolamine, rather than an anxiolytic effect, probably produces 'a situation- and response-dependent alteration in mechanisms of selective attention.' However, given the context of a largely cognitive theory of anxiolytic drug action as developed here, this distinction is not entirely clear-cut.

In the elevated plus-maze test of anxiety, by contrast, scopolamine produces an anxiogenic effect and this extends to an increase in risk assessment in the 'ethological' form of the test (Rodgers and Cole 1995). This is consistent with the view that hypocholinergia may be a crucial component 'of certain psychiatric disorders, such as schizophrenia, post-traumatic stress disorder, and, potentially, depression' (Markou *et al*. 1994).

In relation to its possible interactions with the defence system, scopolamine can block fear conditioning to a tone while leaving conditioning to context intact, whereas the reverse effect is produced by hippocampal lesions and conditioning of both sorts is affected by amygdala lesions (Young *et al.* 1995). Any direct cholinergic contribution to defence must, therefore, be quite restricted.

Conditional delayed discriminations are particularly sensitive to anticholinergics (Kirk *et al.* 1988; Kirkby *et al.* 1995). In these tasks, anticholinergics show a quantitative, but not qualitative, difference from anxiolytic drugs (Tan *et al*. 1990). The deficits produced by anticholinergics are not (as is often reported) in memory decay, but (provided appropriate analysis is used) can be shown to be present at the shortest delays. With delayed matching-to-position, anticholinergic injections into the prefrontal cortex produce delay-independent effects, but injections into the hippocampus produce delay-dependent effects (Dunnett *et al.* 1990). However this latter result could have been due to ceiling effects and the failure to use signal detection measures or to fit an exponential decay curve (see Chapter 8, p. 166).

There has been disagreement as to the precise conditions under which anticholinergics produce memory impairments. Discrepancies have 'been attributed to a variety of factors, including the degree of training, complexity of the task, and dosages of drugs, with anticholinergics causing a greater disruption in partially trained rather than well trained animals, in more complex tasks, and at higher dosages. Moreover, anticholinergics may also reflect nonassociative or performance effects, such as interference with attentional or motivational processes' (Lydon and Nakajima 1992, p. 645). In particular, working memory errors can be produced by anticholinergics

throughout training, whereas reference memory errors are observed only after more extensive training to a higher criterion of performance.

A10.4.2.1 The posterior cholinergic system

The posterior system comprises outflow from both the PPT and LDT. However, the bulk of the literature has focused on the PPT. Except where otherwise indicated, what follows is based on the review of the PPT by Steckler *et al*. (1994*a*).

While the PPT has been associated with motor function, arousal, and sleep, Steckler *et al*. (1994*a*, p. 303) raise the possibility 'that the PPT plays a role in processes of learning and memory' through its influence on the anterior and reticular thalamus. The PPT has some involvement with the control of motor behaviour, with pain, with feeding and sexual behaviour, and to some extent with arousal. In each of these cases activation of the PPT influences the behaviour, but PPT lesions do not produce major changes. For example, PPT lesions block the catalepsy induced by morphine, while themselves producing only minor increases in motor control (Olmstead and Franklin 1994). The PPT also mediates a number of effects of reward in non-deprived rats (e.g. Stefurak and Van der Kooy 1994).

These influences of the PPT on basic maintenance processes make it difficult to assess higher processes. Indeed, 'to date, most studies dealing with PPT's role in cognition have ignored non-specific behavioural influences' (Steckler *et al*. 1994*a*, p. 309). Lesions of the PPT have been found to impair learning in a variety of spatial tasks, suggesting that this may be the system that mediates the effects of scopolamine in such tasks. The tasks affected include an eight- but not a four-arm maze, leading to the proposal that the PPT is involved in sustained attention. However, a direct test of the involvement of the PPT in sustained attention using signal detection measures suggested that the PPT plays a role in the setting of stimulus sensitivity. This suggestion is consistent with the effects, described above, of scopolamine on stimulus sensitivity in delayed conditional discrimination. The effects of PPT lesions on delayed conditional discrimination have not been tested. However, unlike lesions to one of its projection areas, the anterior thalamus, lesions of the PPT do not markedly alter delayed non-matching-to-position (Steckler *et al*. 1994*b*). The PPT appears, therefore, to have only a limited role in the control of stimulus sensitivity and the capacity to perform delayed discriminations. Lesions of the PPT, nonetheless, follow the hippocampal pattern (Appendix 8) of producing impaired passive avoidance with intact active avoidance and increased open field activity. All these effects could be mediated by the direct connections of the PPT to the basal forebrain cholinergic systems.

Finally, recall that relays through the superior colliculus, substantia nigra, and amygdala may play a role in the control of the hippocampal theta rhythm (as discussed in Appendix 5), as may the return connections from these structures to the PPT (see, for example, Fig. 12.8 in Kapp *et al*. 1991).

A10.4.2.2 The NBM/PO complex

In contrast to the hesitation shown by most authors in ascribing cognitive functions to the posterior cholinergic systems, much of the work on the NBM/PO complex has

focused on memory. The bulk of the work has been concerned with the NBM itself. This interest arises from the amnestic effects of anticholinergic drugs, coupled with the fact that basal forebrain cholinergic loss is a consistent feature of senile dementia of the Alzheimer type. In reviewing the data (and see the next section), Kesner and Johnson (in press) note, however, that 'the best support, thus far, for a strong cholinergic influence on memory function is for the MS and VNDB and their projections to the hippocampal formation . . . Support for a strong cholinergic influence of the NBM on memory function is mixed.' With respect to the NBM projection to the dorso-lateral frontal cortex, there are parallels in the patterns of deficit seen after NBM and dorsolateral frontal cortex lesions, respectively, in a duration timing task, but a dissociation in an order recognition memory task. 'Furthermore, with the exception of one study [in the water maze], there are usually no significant correlations or at times negative correlations between depletion of ChAt in cortex following NBM lesions and memory performance. . . . With respect to the NBM projection to parietal cortex and amygdala there are again some parallel patterns of deficits between NBM and parietal cortex in the acquisition of spatial navigation and tactile discrimination learning and between NBM and amygdala in passive and active avoidance learning as well as order recognition memory, but there are also dissociations between NBM and parietal cortex on item recognition memory and NBM and amygdala on taste aversion learning' (Kesner and Johnson, in press).

Consistent with the poor correlations described by Kesner and Johnson, less cholinergically selective methods of lesioning produce more extensive behavioural effects (Robbins *et al*. 1989; see also Connor *et al.* 1991; Riekkinen *et al.* 1991; Steckler *et al*. 1996), and selective damage of cholinergic systems in the NBM has minimal effects on spatial learning and delayed matching-to-place (Baxter *et al*. 1995). Such lesions also have only modest effects on sleep patterns and on the production of hippocampal theta rhythm of all types (Bassant *et al*. 1995). However, the effects of excitotoxic lesions of the NBM, or loss of NBM cells after chronic alcohol ingestion, on spatial learning are substantially reduced by intracortical embryonic grafts with cholinergic characteristics but not by grafts lacking such characteristics (Arendt *et al*. 1989; Hodges *et al*. 1991*a*,*b*; Winkler *et al*. 1995); the effects of NBM lesions can be reversed by systemic administration of cholinergic agonists and exacerbated by cholinergic antagonists (Ridley *et al*. 1986; Hodges *et al*. 1991*a*,*c*; Waite and Thal 1995); and more extensive cholinergic damage, including the MS/DBB, has dose-related effects on spatial learning and passive avoidance (Arendt *et al*. 1989; Leanza *et al*. 1995; see also Hepler *et al*. 1985). These data suggest that the observed effects depend less on the site of the lesions than on the overall loss of acetylcholine (see also Appendix 5). They also strongly suggest that the cholinergic signal is modulatory rather than carrying specific information; and, as we argue in detail in relation to the control of theta rhythm (Appendix 5), that the cholinergic signal may need to be combined with parallel input to target structures from non-cholinergic cells arising in the same basal forebrain nuclei.

As against the thrust of the above data, there is also evidence that the specific region of loss of acetylcholine can play an important role. Different neurotoxins injected into the NBM can deplete the cortex or amygdala, respectively, of acetylcholine. For some memory tasks it is amygdala rather than cortical depletion that is critical (Beninger *et al*. 1994; Mallet *et al*. 1995).

A10.4.2.3 The MS/DBB complex

In contrast to their hesitation about the mnemonic role of the NBM, Kesner and Johnson (in press) conclude that 'the best support, thus far, for a strong cholinergic influence on memory function is for the MS and VNDB and their projections to the hippocampal formation. This is primarily based on the observation of parallel patterns of memory impairments in animals with MS or hippocampus lesions and a positive relationship between lesion size of MS with degree of memory impairment. However it should be noted that other neurotransmitters (e.g. GABA) might also contribute to memory function.' This latter caution is particularly important since, as they note earlier, only one-third or less of medial septal cells are cholinergic.

There are problems, however, in this analysis of the cholinergic contribution of the MS/DBB. Electrolytic lesions provide the main basis for the septal-hippocampal parallels referred to by Kesner and Johnson (see Appendix 8). These have major effects on fibres of passage (including noradrenergic and serotonergic fibres, which may act synergistically with cholinergic cells, see below). Even neurotoxic lesions may damage twice as many non-cholinergic as cholinergic neurons.

Neurotoxic lesions of the MS/DBB can, nonetheless, provide useful information. In particular, if they produce substantial loss of hippocampal acetylcholine with *no* behavioural effect, this would tend to rule out a major role of acetylcholine in that task. However, in general, such lesions tend to have effects similar to those of hippocampal damage (e.g. Ridley *et al.* 1988*a*,*b*; Riekkinen *et al.* 1990*a*). However, even large neurotoxic lesions of the NBM and MS/DBB can leave a variety of memory tasks in monkeys intact, despite having detectable effects on sustained attention (Wenk 1993; Voytko *et al*. 1994).

Systemic scopolamine impairs spatial alternation. This effect is mimicked by intraseptal scopolamine and reversed by intraseptal carbachol (Givens and Olton 1995). This, and other experiments showing that 'direct infusion of scopolamine into the [MS/DBB] impairs both spatial and nonspatial working memory' (Givens and Olton 1995, p. 269), is probably the best evidence we have for a specifically cholinergic involvement in memory tasks.

A10.4.3 Overview of the ascending cholinergic systems

The data we reviewed on systemic drugs were largely based on the effects of muscarinic as opposed to nicotinic agents. However, if we take as a working assumption that cholinergic systems are all involved in a single general class of function, then the muscarinic data are the best available indication as to the nature of that function. Where the effects of muscarinic antagonists are matched by a lesion of one or another component of the ascending cholinergic systems, then we have a chance of drawing more specific conclusions. A failure of cholinergic lesions to reproduce the effects of antimuscarinic drugs, however, could on occasion be due to peripheral actions of the latter (but in most of the critical cases this possibility can be ruled out, since antagonists that act only peripherally, such as methylscopolamine, do not have an effect). An alternative possible explanation of such failures is that an insufficiently large lesion has been made (reported depletions of cortical acetylcholine are frequently of the order of only 50 per cent).

One of the earliest generalizations about the systemic actions of the anticholinergics was that they produced a form of response disinhibition. In this they are very similar to anxiolytic drugs. But there are gaps in this pattern. Anticholinergics fail, for example, to increase responding on some intermittent schedules in which anxiolytic drugs are active. Similarly, the anticholinergics have anxiolytic-like effects in some tests, but these are not as great as would be obtained with anxiolytic drugs, while in others they have anxiogenic-like effects.

More recently, the possible role of cholinergic systems in the control of memory has been the focus of much research. However, the results have again been inconsistent with either a general role in all memory or a specific role in certain types of memory. Rather, cholinergic systems appear to play a critical role in complex tasks during the early phases of training or at higher levels of difficulty. These are all dimensional rather than categorical factors, and there is some indication that the precise parameters required to demonstrate a deficit vary with the dose of the anticholinergic drug.

It is difficult enough to reconcile anxiolytic with amnestic action (although we try to do so in Chapters 9 and 10), but to reconcile an anxiolytic/anxiogenic mixture of actions with a variable amnestic action appears impossible at the level of higher processes. However, this problem may well have arisen because we have so far chosen too high a level of analysis.

A recurring theme in comments on the effects of anticholinergics has been the possible involvement of selective attention or some aspect of arousal. Thus, Rodgers *et al*. (1990), having focused on the possible role of cholinergic systems in defence, concluded that the rather small effects they observed were probably due to an alteration in selective attention rather than to any effect specific to defence. Thus, the 'anxiogenic' effects in the plus maze may be due to an alteration in selective attention interacting with a rather different stimulus situation. Similarly, the effects on 'memory' can be shown, at least in some cases, to be independent of delay. Again, then, the problem may lie in something akin to selective attention.

We argue in Appendix 5 that cholinergic input to the hippocampus 'gates' the theta rhythm. That is to say, in the presence of acetylcholine, the hippocampus becomes more sensitive to the barrage of phasic impulses from the septum. A similar view has been built up in relation to the pyriform cortex by Hasselmo and Bower (1992; Hasselmo *et al.* 1992). As summarized by Hasselmo and Bower (1993), cholinergic input to the olfactory cortex produces a partial suppression of neurotransmitter release from intrinsic fibres, while having no such effect on extrinsic afferent fibres. Concurrently, cell excitability is increased, primarily by 'suppression of voltage- and Ca^{2+}-dependent K^{+} currents, thereby reducing the adaptation of firing frequency in response to sustained depolarization' (Hasselmo and Bower 1993, p. 221). Such a change would increase the possibility of long-term potentiation of the extrinsic input. As one would expect, therefore, antimuscarinics can reduce LTP (Watanabe *et al*. 1995); and, conversely, long-term (at least 20 min) application of a cholinergic agonist at relatively high concentration can induce LTP-like changes, even in the absence of stimulation of the potentiated pathway (Auerbach and Segal 1994).

Here, then, is a potential link with memory. But it is not a direct link. In the model presented by Hasselmo and Bower, in any particular part of a neural network an initial

memory can be formed adequately without the need for cholinergic input, since only extrinsic fibres would be active. It is when a second memory needs to share part of the same network that a problem could arise. In the absence of cholinergic input, intrinsic connections established in the formation of the first memory can become active, resulting in the strengthening of inappropriate connections, and so essentially confounding the two memories. In the presence of cholinergic input, the intrinsic connections are dampened and the two memories can be kept discrete.

This model accommodates many of the facts concerning the involvement of cholinergic systems in memory: their importance in early rather than later stages of learning; the influence of task complexity; etc. Note, however, that it is a model of the role of acetylcholine in systems whose function is precisely that of forming memories. There is no requirement for memory formation as such to be the primary role of acetylcholine nor, indeed, for any memory formation at all. Thus, in relation to the hippocampus, acetylcholine release will often be important for non-mnemonic processing, but again under conditions in which there is the same requirement for an increased external signal-to-internal-noise ratio (c.f. Vinogradova *et al*. 1993*b*).

Recently, intracerebral microdialysis has been used to show that acetylcholine is released in response to simple sensory stimuli to which the animal reacts with locomotion (hence showing a form of orienting response). Acetylcholine release was also observed when the animal was regularly placed in an experimental chamber, with a further increase in release when it learned that a reward was available (Inglis *et al.* 1994; Inglis and Fibiger 1995). Relevant to the argument pursued here, these workers found that different stimuli produced different patterns of release of acetylcholine in the cortex and hippocampus. For example, increases in the amount of acetylcholine produced by prior association with reward occurred only in the hippocampus, not the cortex. Thus, the same overall 'attentional'/'signal-to-noise' function may be subserved by all cholinergic systems; but the net effect from the psychological point of view may depend on which parts of the cholinergic systems are active, and on the specific functions of the relevant target structures. The apparent complexity of cholinergic function arises, then, not from its intrinsic complexity, but from the fact that the same basic neuronal modulation can result in, for example, memorial change in a 'memorial' part of the brain and attentional change in an 'attentional' part.

Let us look again at the different components of the cholinergic system in the light of these considerations.

The literature contains the following suggestions, reviewed to varying degrees above. With regard to the *posterior cholinergic system*, the effects of both lesions and stimulation have often been taken to suggest a role in some aspect of arousal or attention. With regard to the *NBM/PO system*, the emphasis has been on a memorial function (although the evidence in favour of this view is in fact stronger in relation to the MS/DBB complex). To a large extent, the data on the NBM/PO system suggest that loss of cholinergic input impairs the functioning of relevant target areas. Thus the effects of NBM/PO lesions are largely similar to those of lesions to the frontal cortex, parietal cortex, and amygdala. As to the *MS/DBB complex*, again it appears that the effects of lesions are to impair the function of its target areas (mainly the hippocampal formation).

Given the topographic distribution of cholinergic connections and the fact that, in general, the effects of the different types of lesion depend on the functions of their targets, we can ask how far the cholinergic systems are in fact separate one from another. In Appendix 5 we demonstrate that the effects of activation of the PPT on the hippocampal theta rhythm are mediated by a system which first diverges to innervate areas as disparate as the superior colliculus and substantia nigra, and then converges (via non-cholinergic elements) probably on the thalamus, before being relayed by the septum to the hippocampus. Thus, for at least this one highly specific aspect of brain function, the posterior and MS/DBB systems are essentially a single unit, probably accounting for the role of the PPT in behavioural inhibition. However, the projections of the PPT to, for example, the substantia nigra cannot be there solely for the purpose of relay to the hippocampus. It seems likely, indeed, that these projections account for the role of the PPT in motor behaviour.

Taken overall, then, a case can be made for viewing the cholinergic systems as a reticulum. All the cells would have the same basic function, and often the capacity to excite each other so that activation of one part of the reticulum would tend to spread to other parts. The cholinergic outflow would then increase signal-to-noise ratio (particularly, extrinsic relative to intrinsic sensitivity) in many of its target areas, but with quite different behavioural consequences depending on which target. This arrangement may be comprehensible from an evolutionary point of view, if the various cholinergic areas have progressively differentiated from some primordial cluster. This possibility is consistent with both the diffuse distribution of cholinergic cells (e.g. Armstrong *et al*. 1983) and the fact that cholinergic cells can be found in more than 40 separate areas of the brain (Kimura *et al.* 1981).

This is not to say that the reticulum is undifferentiated; indeed, as we have noted, there seems to be considerable topographic organization. Nor is it to say that the reticulum would always act in a homogeneous fashion. Indeed, the experiments from Fibiger's group described above suggest that different patterns of stimuli release acetylcholine differentially in different parts of the brain. Nonetheless, even in Fibiger's experiments there was considerable coherence in acetylcholine release across areas, and we need not suppose that different parts of the cholinergic reticulum have totally distinct functions.

A10.5 Interactions of the aminergic systems

Proceeding further with this line of thought, one can ask how far the three different aminergic systems have entirely distinct functions. Each appears to be involved in producing some change in the signal-to-noise-ratio in its targets; they all innervate much the same areas of the brain and in much the same way; they all take essentially the same three routes to innervate the frontal cortex, hippocampus, amygdala, and related structures, travelling in much the same fibre bundles; and they all have some form of topographic organization (McNaughton and Mason 1980; Gaykema 1992; Gonzalo-Ruiz *et al.* 1995). Indeed, there is greater anatomical similarity between the three systems, each taken as a whole, than between the individual components of any one system. This pattern suggests that the three aminergic systems may perform largely similar end-functions, but are activated each under somewhat different environmental conditions. Given the advantage to the animal of a seamless integration

of different rules of thumb addressing the same adaptive problem, it would not be surprising to find synergistic interactions between the systems.

Consistent with this suggestion, noradrenergic lesions which do not themselves impair spatial learning greatly increase the effects of anticholinergic drugs (Decker and Gallagher 1987), even when they do not affect the functioning of cholinergic cells. Similarly, noradrenaline in the amygdala produces at least some of its behavioural effects by releasing acetylcholine (Dalmaz *et al.* 1993). Equally, nicotinic activation can increase the firing of locus coeruleus neurons (Engberg and Hajos 1994).

The evidence is even stronger for synergistic interaction between cholinergic and serotonergic systems. Electrolytic septal lesions and 5,7DHT raphe lesions can potentiate each other's effects on spatial learning (Nilsson *et al*. 1988). Even when 5,7DHT raphe lesions do not impair spatial learning, they can potentiate the effects of ibotenic acid lesions of the NBM (Riekkinen *et al.* 1990*b*). A subthreshold dose of atropine or scopolamine combined with a subthreshold depletion of 5-HT can impair acquisition of spatial position (Richter-Levin and Segal 1989; Harder *et al*. 1996). Similarly, scopolamine and the 5-HT_2 antagonist methysergide have been shown to act synergistically (Riekkinen *et al*. 1992; but see also Sakurai and Wenk 1990), as have scopolamine and the 5-HT_{1A} agonist 8-OH-DPAT (Riekkinen *et al*. 1995). Interestingly, the effects of 5,7DHT lesions on spatial learning in the presence of a cholinergic blocker appear almost entirely attributable to the serotonergic innervation of the hippocampus (Richter-Levin *et al.* 1994*a*,*b*).

As might be expected from these findings, 5-HT_4 and 5-HT_3 stimulation in the frontal cortex and hippocampus, respectively, have been shown to release acetylcholine (Consolo *et al*. 1994*a*,*b*). However, there is also evidence for more complicated interactions, in that 5-HT can *inhibit* acetylcholine release from the hippocampus via 5-HT_{1B} receptors (Maura *et al.* 1989); and 5-HT_{1A} receptors are found on the cell bodies (and probably dendrites) of 25 per cent of septal cholinergic cells (Kia *et al*. 1996). Given the variety of synergies possible between the systems, it seems likely that there is negative feedback at higher levels (see, for example, Dalmaz *et al*. 1993). This could be achieved if the affinities of the facilitatory receptors for the transmitter were lower than the affinities of the inhibitory ones. However, at least comparing 5-HT_{1B} with 5-HT_3 receptors, this does not appear to be the case (Griebel 1995, Table 1).

There are considerable 'histological, electrophysiological, pharmacological and behavioural data suggesting that serotonin is able to modulate central cholinergic function and that this modulation may have, in some respects, cognitive implications' (review by Cassel and Jeltsch 1995, p. 31). In addition, as we saw in Appendix 5 when discussing the control of theta rhythm, independent but synergistic control of certain processes by 5-HT and ACh is also possible. For our present purposes it is probably only necessary to note that the systems do interact and leave for the future more explicit details.

Finally, we note a study which explicitly compared the firing to the same set of stimuli of 'broad-spike' presumed aminergic cells in the laterodorsal tegmental nucleus, the locus coeruleus, and the dorsal raphe respectively (Koyama *et al.* 1994). These authors found that dorsal raphe neurons displayed tonic increases in firing

which accompanied EMG activation; locus coeruleus cells, by contrast, showed a phasic increase in firing in relation to the presentation of a sensory stimulus, these responses undergoing little habituation. There was little variation in the general type of response within the raphe or locus coeruleus groups of cells. ‘Compared with these, the laterodorsal tegmental neurons were heterogeneous: about one-quarter showing only a simple change of firing (half increasing, half decreasing); and two-thirds displaying phasic responses. The latter responses of many neurons attenuated strongly upon repetition’ (Koyama *et al.* 1994, p. 1021). There was no obvious relation between the pattern of response shown by any cell during waking and its pattern of firing during sleep.

These data suggest that ‘the rapid cholinergic system controls the general condition of the brain (including sleep and wakefulness), cooperating with the "slow" noradrenergic and serotonergic systems. The three systems, which may interact mutually, may share the function of "the ascending reticular activating system"’ (Koyama *et al.* 1994, p. 1030).

A10.6 Conclusions

From the above we conclude that any role the aminergic systems play in defence is indirect. However, this is not to say that this role is unimportant.

When we make a direct comparison (Table 4.2), there are surprisingly extensive parallels between the behavioural effects of cholinergic suppression and those of septo-hippocampal lesions. Some parallel would of course be expected given the MS/DBB cholinergic input to the hippocampus, but the relative lack of parallel between cholinergic blockade and lesions to the other terminal areas is surprising. This lack of parallel is not total, in that changes in active avoidance can be attributed to the cholinergic input to the amygdala.

If we lump together lesions to the noradrenergic and serotonergic systems, there are remarkably good parallels with septo-hippocampal damage. Each system appears to contribute part of the septo-hippocampal syndrome. More so even than with the cholinergic system, there is a surprising lack of effects corresponding to those of lesions to the other terminal areas innervated by these systems.

Our best explanation of these discrepancies is that, hypothetically, the hippocampus, more than any other area, requires a critical minimum of input from all three systems, so that damage to any one has functional consequences. Even so, with heavily ‘hippocampal’ tasks such as the water maze, combined loss of two or more of the aminergic inputs is more deleterious than loss of one only. For the other areas of the brain, therefore, we may postulate that lesion of only one aminergic pathway is insufficient to affect behaviour significantly, as the systems are effectively redundant. On this view, the behavioural profile of combined selective lesions to all three systems should result in major changes, many of which would *not* be characteristic of hippocampal damage.

Combining all the data together, we can speculate that activity in the serotonergic system is the result of an efference copy of priming of repetitive motor programmes

designed to cope with regularly occurring circumstances; by contrast, activity in the noradrenergic system is a consequence of priming of phasic motor programmes, particularly orienting responses; and the cholinergic system shares properties of both of these systems, coming into action whenever information related to the priming of motor programmes is required to be processed in a precise fashion. In none of these cases is the system supposed to be involved in the control of those systems which activate it.

In many cases, the aminergic systems will be activated at times when they have no functional effect on behaviour. Thus, each system will alter signal-to-noise ratios. However, the serotonergic system will be more concerned to prevent the dominant motor programme (e.g. avoidance) from being interrupted by concurrent activation of some other motor programme (e.g. escape); the noradrenergic system will be more concerned to prevent the dominant controlling stimulus from having its control of behaviour interrupted by other concurrent stimuli; and the cholinergic system will be more concerned to prevent the current-to-be-associated stimulus from having its associative connections interrupted by other concurrently activated associations (Vinogradova *et al*. 1993*a*). These increases in signal-to-noise ratio will have functional effects only if the target structure is processing a signal and if the result of that processing is a functional output.

In achieving these different effects, we can assume that the aminergic systems produce largely similar direct neural effects, but produce their different patterns of response through requiring different adequate stimuli for their activation. We can also assume that the fundamental effect of release of transmitter by the systems is not only similar between them but very simple. The apparent complexities of the effects of drugs and lesions are then attributed to the complexities of the functions of the various target areas.

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Notes

1. Lesion of the DANB reduces the occurrence of LTP in the hippocampus; see Bliss and Lynch (1986).

2. This prediction arose from a discussion with Dr L. Stein at a symposium held at the Eastern Psychological Association in Philadelphia in 1979.