# GENERAL DISCUSSION

The results of the four experiments presented in this thesis suggest that disruption of 5-HT function can produce anxiolytic-like effects in the elevated plus-maze, and to a lesser degree, in the shock-probe burying test. In Experiment 1, 5-HT was depleted using the 5-HT synthesis inhibitor, p-CPA. Compared to saline-injected control rats, rats treated with p-CPA showed significant increases in open-arm activity in the plus-maze test, and a substantial, but non-significant decrease in burying behaviour in the shock-probe test. These effects are similar to those produced by anxiolytic drugs in these same tests. In Experiments 2, 3, and 4, 5-HT function was disrupted in the brain using electrolytic lesions of 5-HT-containing cell bodies of the midbrain raphe nuclei. Compared to sham lesions, destruction of the dorsal raphe (Experiments 3 & 4), or of the dorsal and median raphe combined (Experiment 2), produced significant anxiolytic-like increases in open-arm activity in the plus-maze. Lesions of the median raphe alone (Experiment 3) did not produce a significant anxiolytic effect in the plus-maze test. In the shock-probe test, combined DR and MR lesions produced a non-significant decrease in probe burying in Experiment 2, but in Experiment 3, DR lesions alone had no effect on burying, and MR lesions actually increased (non-significantly) the duration of

burying. In spite of these inconsistencies, DR lesions in Experiment 4 produced significant anxiolytic effects in the shock-probe test.

The results of these four experiments provide some support for the view that 5-HT disruption produces anxiolysis in animals. Although earlier research utilizing the conflict model also suggested that 5-HT disruption could produce anxiolytic-like effects in rats (see introduction), these effects were often inconsistent, and were confounded with the effects of 5-HT depletion on thirst, hunger, and possibly learning mechanisms. It was argued that the use of simpler models of experimental anxiety, based on animals' untrained fear reactions to aversive (painful or novel) stimuli, might provide clear, consistent evidence that 5-HT disruption produces anxiolytic-like effects. Both the plus-maze test and the shock-probe test are based on such untrained fear reactions, are sensitive to anxiolytic drugs, and do not involve food or fluid reward. The combined use of these two tests was particularly important, since the index of anxiolysis in each test is distinct: the index of anxiolysis in the plus-maze test is an increase in behaviour (open-arm activity), while the index of anxiolysis in the shock-probe test is a decrease in behaviour (probe burying). Thus, it was argued, if anxiolytic profiles were observed in <u>both</u> tests after 5-HT manipulations, the results would be difficult to explain in terms of non-specific effects on

general activity, arousal, or behavioral inhibition. However, this ideal pattern of results was only approximated in the present experiments. Although 5-HT-depleted rats showed a reliable increase in open-arm activity in the plus-maze test, they did not show reliable and consistent decreases in burying behaviour in the shock-probe test. This inconsistency in the overall pattern of results is problematic for the hypothesis that anxiety reduction is mediated by 5-HT reduction. In the following sections, the inconsistencies in the experimental results will be discussed in more detail, and alternative interpretations of the data will be presented.

Once again, with the exception of localized MR lesions, every manipulation used to reduce 5-HT function (p-CPA, lesions of both DR and MR, or of the DR alone) produced a clear and significant anxiolytic profile in the plus-maze test. By itself, this set of plus-maze data suggests that 5-HT reduction produces robust anti-anxiety effects in rats. In contrast, animals with complete DR lesions in Experiment 4 showed a significant reduction in burying behaviour in the shock-probe test. Animals given combined DR and MR lesions (Experiment 2), or p-CPA treatment (Experiment 1), showed non-significant decreases in burying behaviour. Animals with lesions of the DR in Experiment 3 showed no change in burying behaviour, and animals with MR lesions showed non-significant increases in burying behaviour.

There are a number of possible factors which could have contributed to these inconsistencies. One factor which could have contributed, was differences in the baseline variability inherent in the two tests. In general, the plus-maze test elicits fairly stable baselines of responding, with control animals showing a mean open-arm activity level between 10-20 percent. In addition, the variability (SEM) of open-arm activity within a group is usually less than 10% (see Figures 1, 3, 6, and 9). In contrast, the mean duration of burying exhibited by control animals in the shock-probe test can be quite variable (eg., from 50 seconds in Experiment 3, to 126 seconds in Experiment 2) and the variability (SEM) within groups is usually 30 seconds or more (see Figures 2, 4, 7, and 10). Since defensive burying behaviour is a somewhat more variable measure than open-arm activity, it is possible that the shock-probe test would be less sensitive to changes in 5-HT levels than the plus-maze test. If this were the case, we might expect to see a more pronounced, significant pattern of results after 5-HT disruption in the plus-maze test than in the shock-probe test. The results of the present experiment confirm this expectation. The plus-maze test produced consistent, significant anxiolytic-like effects in nearly all cases of 5-HT depletion, while the shock-probe test produced non-significant anxiolytic trends in most cases, and a significant anxiolytic effect in only

one case (DR lesions in Experiment 4).

In spite of possible differences between the plus-maze test and the shock-probe test in terms of baseline variability, both tests appear to be equally sensitive to the effects of anxiolytic drugs. In fact, the anxiolytic effect of diazepam has been detected in the shock-probe test at doses as low as 0.2 mg/kg (Rohmer, Di Scala, & Sandner, 1990), whereas slightly higher doses (1.0 mg/kg) are required to produce anxiolysis in the plus-maze test (Pellow et al., 1975). Considering this, it does not seem likely that two tests almost equally sensitive to the effects of diazepam, would be differentially sensitive to the "anxiolytic" effects of 5-HT depletion.

Another possibility is that some secondary 5-HT mediated effect might have counteracted the expression of anxiolysis in the shock-probe test, but not in the plus-maze test. Disruption of 5-HT in the brain has been shown to alter pain sensitivity in animals, generally increasing the sensitivity to pain (Hamon et al., 1990). In addition, 5-HT neural systems appear to be involved in inhibiting a pain pathway to the thalamus (e.g., Preito-Gomez et al., 1989). Considering this, it is likely that shock-motivated models of anxiety, such as the shock-probe test, might be complicated by 5-HT-induced changes in pain sensitivity, while models which do not employ painful stimuli, such as the plus-maze test, would not be affected. Perhaps the

failure of the shock-probe test to reveal consistent anxiolysis was a result of a concurrent increase in pain sensitivity, which masked a 5-HT reduction-induced anxiolytic effect. However, if increased pain sensitivity were responsible for antagonizing the anxiolytic effects of 5-HT manipulations, it might be expected 5-HT-depleted animals would either be more reactive to shock, would take fewer shocks, or might possibly even bury the probe longer than normal animals.

The results from the shock-probe test are clearly not consistent with these expectations. Animals treated with p-CPA (Experiment 1) did not show significant differences compared to saline-injected controls in their reactivity to shock, or in the number of probe contacts made, but did show a reduction in their duration of burying (see Figure 1 & Table 2). Similar results were found in animals with combined lesions of the DR and MR (see Table 3). In Experiment 3, DR-lesioned animals showed a decrease in shock reactivity, but no suppression of burying, and no significant difference in the number of probe contacts made. In Experiment 4, animals sustaining DR lesions showed a significant suppression of burying, but no difference in shock reactivity or number of probe contacts. In none of the four experiments in this thesis was there direct evidence of increased pain sensitivity in 5-HT depleted animals. In order to assess whether increased pain

sensitivity indeed acts to counteract the suppression of the burying response in 5-HT-disrupted animals, it would be necessary to investigate pain sensitivity in independent tests of analgesia (i.e., the tail-flick test).

Changes in general activity induced by 5-HT disruption might also introduce variability into the behavioral data, theoretically either mimicking or masking the expression of an anxiolytic profile in rats. Hyperactivity has often been observed in animals after 5-HT disruption (e.g., Fibiger & Campbell, 1971; Marsden & Curzon, 1977). Casual observation in the present experiments revealed that animals given p-CPA, MR lesions, or combined MR and DR lesions, did exhibit some general behavioral agitation in their home cages, whereas animals given DR lesions did not. These observations are compatable with evidence suggesting hyperactivity is due to disruption of 5-HT in the MR (Azmitia, 1975; Kostowski, Giacalone, Garattini, & Valzelli, 1968). Despite these observations, however, no other evidence of hyperactivity was observed during handling of rats in the present experiments, and more importantly, no hyperactivity was seen in either the plus-maze or the shock-probe tests of anxiolysis. Increases in the number of arm entries in the plus-maze were seen after 5-HT disruption, but this increased activity was always selective for the open arms of the maze.

In the case of the shock-probe test, hyperactivity

could theoretically either potentiate or interfere with the burying response. However, the only significant change in general activity seen in this test occurred in Experiment 1. Rats treated with p-CPA showed a significant decrease in activity, which was correlated with with a (non-significant) decrease in burying. In this case, the decrease in burying behavior might have been secondary to an overall decrease in general activity. In any case, there was no evidence that hyperactivity affected animal behaviour in these tests.

Alternatively, it is possible that some of the variability in the effects seen in the plus-maze and the shock-probe tests was related to an effect of 5-HT disruption on the ability of animals to inhibit responding. There is some evidence which suggests that low brain levels of 5-HT might be related to increased agressiveness in rhesus monkeys, while high levels of 5-HT are related to timidity (Higley et al, 1990). Additionally, in a standard operant paradigm, p-CPA administration increased resistance to extinction at the end of a continuous reinforcement schedule in rats (Rosen & Freedman, 1974). Furthermore, electrolytic lesions of the raphe in rats impaired performance on a schedule of reinforcement for low response rates (Wing & Wirtshafter, 1982). These studies suggest 5-HT depletion might disrupt animals' ability to inhibit responses that are no longer rewarded, or responses that lead to reward reduction. It has been proposed that this

"disinhibition" of behaviour is the source of what appears to be anxiolysis in conflict tests, where animals must inhibit punished responding (Soubrie, 1986). It is possible that general "disinhibition" induced by 5-HT reduction could have contributed to the results seen in the plus-maze test in the present experiments. If the inhibition of open-arm exploration in normal animals is maintained by 5-HT, animals with reduced 5-HT function would be expected to show a "disinhibition" of open-arm exploration. The results of the present experiments are in fact consistent with this explanation.

Despite this, the concepts of "conflict" and "disinhibition of behaviour" cannot be easily applied to the shock-probe test. To begin, defensive burying is not inhibited by the "fearful" shock stimulus. Instead, it is elicited by the shock. It is therefore difficult to see how disinhibition applies to this measure of anxiety. Any factor which produces a general behavioral "disinhibition" would not necessarily be expected to alter the burying response. In fact, with one exception, defensive burying was not significantly affected by the 5-HT manipulations used in these experiments. In a sense, this dichotomy of results in the plus-maze and shock-probe tests after 5-HT depletion, favors the hypothesis that 5-HT reduction produces a general response disinhibition: Apparent "anxiolytic" effects were reliably observed in the plus-maze

test as an increase in (inhibited) open-arm activity, but were not reliably observed in the shock-probe test as a decrease in (non-inhibited) burying.

However, some data from the shock-probe test are inconsistent with the "disinhibition" interpretation. A disinhibited animal might be expected to approach the probe more often, and thereby receive more shocks. The shock-probe test data do not support this expectation. None of the 5-HT depleted rats showed an increase in the number of shocks received, except animals with MR lesions (Experiment 3), and these experimental animals were the only ones that did not show any significant increase in open-arm activity in the plus-maze test. Thus, while it is possible that "general disinhibition" might be related to some of the variability in results seen in the two tests of anxiolysis in these experiments, this idea cannot account for the entire pattern of results.

It is possible that the effects of 5-HT depletion on animals' learning ability might influence animal responding in the shock-probe test. Electrolytic lesions of the raphe have been shown to impair learning of a one-way avoidance response (Hole & Lorens, 1975), and to impair stimulus discrimination ability in rats (Wirtshafter et al., 1983). Administration of p-CPA has been shown to disrupt latent inhibition in rats (Solomon, Kiney, Scott, 1978). In the plus-maze test of anxiolysis, animals avoid the open maze

arms without any prior experience or training, and do not need to learn any associations between stimuli and responses. In the shock-probe test, animals bury the shock source without prior training or experience, but some learning of an association between the probe and electric shock must occur before animals can accurately direct the burying response toward the probe (Pinel & Treit, 1978). Thus, neural manipulations that disrupt associations of stimuli with aversive consequences might be expected to disrupt burying behaviour directed toward the probe.

In the four experiments discussed in this thesis, most 5-HT depleted rats showed non-significant decreases in the duration of burying. While this trend is consistent with a disruption in associative learning ability in these animals, there are two problems this interpretation. First, other studies (e.g., Hole & Lorens, 1975) investigating raphe lesion effects on learning ability suggest that MR-lesioned animals have the greatest learning deficits. However, the results of the present experiments show that MR-lesioned animals actually buried the probe more, not less, than control animals. Second, with one exception, 5-HT-depleted animals were able to avoid contact-induced shocks from the probe at least as well as control animals. This would not be expected if these aimals were unable to learn an association between the shock and the probe. Thus, it is unlikely that 5-HT depletion produced the behavioral effects

seen in the shock-probe tests through a disruption of associative learning.

Although it has been assumed that the behavioral effects seen in these experiments were due to a disruption of 5-HT, it is possible that some of these effects were a result of alterations of neurotransmitters other than 5-HT. However, histological analysis of raphe-lesioned animals revealed no damage to the locus ceruleus or substantia nigra, areas containing high densities of catecholamine neurotransmitters (NE and DA, respectively). While it is possible that electrolytic lesions of the raphe might have marginally or indirectly affected NE or DA, the behavioral results from these experiments were not due to direct damage to the locus ceruleus or substantia nigra. Furthermore, the behavioral effects of electrolytic lesions of the raphe generally paralleled the behavioral effects of p-CPA treatment, which produces a very selective depletion in 5-HT when administered under the dose schedule used in Experiment 1 (Koe & Weissman, 1966). For these reasons, it is unlikely that catecholamine disruption can account for the behaviours seen in these experiments. Nevertheless, definitive answers to these questions must await similar studies which include concurrent brain assays of neurotransmitter systems.

5-HT depletion and anxiolysis: Regional specificity. If the magnitude of anxiolysis in animal models was inversely proportional to the magnitude of 5-HT depletion in the brain, we might expect that p-CPA treatment would produce the most dramatic anxiolytic effects in rats, followed by combined DR and MR lesions, and then by individual lesions of either the DR or the MR. This theoretical pattern of results is incongruent with the results from the present experiments, however. In fact, it could be argued that 5-HT in the DR was critical in mediating experimental anxiety in the present experiments. Although previous research suggests that electrolytic lesions of either the DR or the MR will reduce total forebrain 5-HT to a similar extent in rats (Jacobs et al., 1974), DR (and not MR) lesions in Experiment 3 produced significant anxiolysis in the plus-maze test, and in Experiment 4, DR lesions produced clear anxiolysis in both the plus-maze and the shock-probe tests. Furthermore, there was some histological evidence that more complete DR lesions were associated with more complete anxiolytic effects. In contrast, electrolytic lesions of the MR failed to produce clear evidence of anxiolysis in either the plus-maze or the shock-probe tests.

The MR has been shown to project to the DR (Aghajanian & Wang, 1978). Since there are 5-HT autoreceptors in the DR which inhibit 5-HT release (Moret, 1985), these MR connections may serve to inhibit the DR, reducing 5-HT

transmission to the forebrain. If this is the case, it seems reasonable that once the inhibitory influence of the MR is removed, the resultant release of DR inhibition might actually increase anxiety by making more 5-HT available to the forebrain. In other words, MR lesions might be "anxiogenic" rather than anxiolytic. It is important to note, however, that although MR-lesioned rats in Experiment 3 showed an increase in burying compared to controls, they did not show a corresponding anxiogenic decrease in open-arm activity in the plus-maze test. Further investigation of the effects of MR lesions on animal "anxiety" is necessary before any conclusions can be made regarding it's role in mediating experimental anxiety. However, it is possible this factor contributed to some of the variability seen in this set of experiments.

Although it has been argued that the DR (and not brain 5-HT in general) is critical in mediating experimental anxiety, there is the problem of the discrepancy between the results obtained after DR lesions in Experiment 3 and in Experiment 4. Lesions of the DR in Experiment 3 produced a significant increase in the percent time in the open arms of the plus-maze, but only a non-significant increase in the percent of open-arm entries. In the shock-probe test, lesions of the DR in Experiment 3 failed to produce any effect on the duration of burying, but lesions of the DR in Experiment 4 produced robust and significant anxiolytic

effects on defensive burying in the shock-probe test, and on both measures of open-arm activity in the plus-maze test. If the DR-lesioned groups in Experiment 3 and 4 received the same lesion, behavioral data should not have differed so greatly between these experiments. However, close inspection of the histologies from the two DR-lesioned groups showed that they did not sustain an equivalent degree of damage to the target site. There were several differences between DR lesions in Experiment 3 and those in Experiment 4. Although both DR groups met the criteria for an acceptable DR lesion, animals with DR lesions in Experiment 3 appeared to sustain more extra-raphe damage within 0.5 mm of the DR than did animals with DR lesions in Experiment 4. More importantly, however, was the difference in the amount of DR tissue actually lesioned. In Experiment 3, animals tended to sustain lesions that spared much of the anterior portion of the DR. Failure to lesion the entire DR, especially the anterior portion spares much of the 5-HT transmission to the forebrain (Lorens & Guldberg, 1974). In Experiment 4, however, the DR lesions were usually complete. Considering this difference in the DR lesions in these experiments, it is possible that the difference in degree of anxiolysis seen in these animals might reflect differences in the degree of 5-HT disruption in the DR. This idea suggests that 5-HT in the DR mediates "anxiety" in animals. Nevertheless, although animals with

more complete lesions of the DR in Experiment 4 showed more dramatic anxiolytic behavioral profiles in both tests of experimental anxiety, animals with complete lesions of the DR (combined with MR damage) in Experiment 2, showed significant anxiolysis in only the plus-maze test, and not in the shock-probe test. This inconsistency is difficult to reconcile with the hypothesis that complete DR lesions lead to complete anxiolysis, although it is possible that the additional damage to the MR in Experiment 2 may have weakened the effects of specific damage to the DR.

The septum-DR link. In spite of the complications discussed previously, the hypothesis that 5-HT disruption leads to anxiolysis in animals has some degree of support, especially from the plus-maze test. While there are still a great number of questions left to be answered, this finding might not be surprising, given that the raphe nuclei send 5-HT projections to a variety of limbic structures, which have long been implicated in emotional behavior (Papez, 1937). These limbic areas include the septum, amygdala, hippocampus, and nucleus accumbens (Consolazione & Cuello, 1982; Gray, 1982; Parent et al., 1981). While 5-HT input into any of these areas might modulate anxiety in animals, the behavioral effects of the posterior septal lesions found in Experiment 4 suggest the connections of the raphe with the septum might be particularly important. Lesions of the

septum have previously been shown to produce anxiolysis in the plus-maze and shock-probe tests (Gray et al., 1981; Treit & Pesold, 1990; Pesold & Treit, 1992). In Experiment 4, lesions of the either the PS or the DR produced similar anxiolytic effects in both tests. This similarity in the behavioral effects of DR and PS lesions may be related to the well known anatomical connections between the DR and the septum (Segal & Landis, 1974). Furthermore, serotonergic neurotoxic lesions of the septum using 5,7-DHT produced anxiolytic-like effects in a social interaction test of anxiolysis (Clarke & File, 1982). In combination, this evidence suggests 5-HT input into the septum might modify anxious behaviour in rats. This evidence is consistent with Gray's (1982) notion that the raphe-septo-hippocampal system of the brain is central to producing anxiety in animals.

It is interesting to note that PS lesions completely eliminated defensive burying in Experiment 4, whereas DR lesions suppressed but did not eliminate this response. This might suggest that the PS is central to anxiolysis in the shock-probe model, and that 5-HT projections from the DR modify the expression of this fear reaction through the septum. It would be interesting to explore this possibility by assessing the effects of 5,7-DHT lesions of the septum in the plus-maze and shock-probe tests.

5-HT and GABA. A final point concerns the possible connection between 5-HT, GABA, and anxiety. There is a great deal of evidence that benzodiazepine anxiolytics exert their behavioral effects through facilitation of GABA neurotransmission in the brain (e.g., Haefely, 1989; Hommer, Skolnick, & Paul, 1987). There is also evidence that GABA stimulation inhibits DR firing in the brain (Gallager, 1978) Manipulations using GABAergic drugs or benzodiazepines in the DR also modify anxiety in animals (Collinge, Pycock, & Taberner, 1983; Green et al., 1983; Higgens et al, 1988; Sainati & Lorens, 1982; Soderpalm & Engel, 1989; Soderpalm & Engel, 1991). It is therefore possible that BZs exert their anxiolytic effects indirectly through an inhibition of 5-HT transmission as a result of facilitating GABA. If this is the case, it seems understandable that either BZ-induced increases in GABA transmission, or direct decreases in 5-HT transmission might produce anxiolysis in rats.

Conclusions. Although these speculations are interesting, none of them offer convincing explanations of the entire pattern of results obtained in the present experiments. The simple idea that a reduction of 5-HT should uniformly lead to a reduction in experimental anxiety was not confirmed by the results of the present experiments. Although the results from the plus-maze test were in agreement with the 5-HT hypothesis, the results of the shock-probe tests were

often inconsistent with this hypothesis.

Thus, the inconsistencies which plagued the previous literature (see introduction) also marred the results of the present experiments. Clearly, further research is needed to show that 5-HT plays a direct and pervasive role in modulating experimental anxiety. Nevertheless, the elevated plus-maze test and the shock-probe burying test appear to be useful models with which to explore the relationship between 5-HT and anxiety.

## References

Aghajanian, G.K. & Haigler, H.G. (1974). Mode of action of LSD on serotonergic neurons. Advances in Biochemical Psychopharmacology, 10, 167-177.

Aghajanian, G.K. & Wang, R.Y. (1978). Physiology and pharmacology of central serotonergic neurons. In M.A. Lipton, A. DiMascio, & K.F. Killam (ed's). Psychopharmacology: A Generation of Progress. New York: Raven Press.

Amin, A.H., Crawford, T.B.B., & Gaddum, J.H (1954). The distribution of substance P and 5-hydroxytryptamine in the central nervous system of the dog. Journal of Physiology, 126, 596-618.

Azmitia, E.C. (1975). Serotonin-producing neurons of the midbrain raphe nuclei. In L.L. Iversen, S.D. Iversen, & S.H. Snyder (ed's), Handbook of psychopharmacology (v. 9, pp. 282-283). New York: Plenum Press.

Azmitia, E.C. & Segal, M. (1978). An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei of the rat. The Journal of Comparative Neurology, 179, 641-668.

Barofsky, A-L., Greir, H.C., & Pradhan, T.K. (1980). Evidence for the regulation of water intake by median raphe serotonergic neurons. Physiology & Behaviour, 24, 951-955.

Beardslee, S.L., Papadakis, E., Fontana, D.J., & Commissaris, R.L. (1990). Antipanic drug treatments: Failure to exhibit anxiolytic-like effects on defensive burying behaviour. Pharmacology Biochemistry & Behaviour, 35, 451-455.

Beitz, A.J. (1990). The anatomy of ascending serotonergic pathways possibly involved in pain modulation. In J.-M. Besson, ed. Serotonin and Pain. New York: Elsevier Science Publishers.

Bendotti, C. & Samanin, R. (1986). 8-Hydroxy-2-(di-n-propylamino) Tetralin (8-OH-DPAT) elicits eating in free feeding rats by acting on central serotonin neurons. European Journal of Pharmacology, 121, 147-150.

Bendotti, C. & Samanin, R. (1987). The role of 5-$HT_{1A}$ and 5-$HT_{1B}$ receptors in the control of feeding in rats. Life Sciences, 41, 635-642.

Blakely, T.A. & Parker, L.F. (1973). The effects of parachlorophenylalanine on experimentally induced conflict behaviour. Pharmacology Biochemistry & Behaviour, 1, 609-613.

Blampied, N.M. & Kirk, R.C. (1983). Defensive Burying: Effects of diazepam and oxprenolol measured in extinction. Life Sciences, 33, 695-699.

Blundell, J.E. & Latham, C.J. (1979). Serotonergic influences on food intake: Effects of 5-hydroxytryptophan on parameters of feeding behaviour in deprived and free-feeding rats. Pharmacology Biochemistry & Behaviour, 11, 431-437.

Bolles, R.C. (1970). Species-specific defense reactions and avoidance learning. Psychological Review, 77, 32-48.

Bradley, P.B., Engel, G., Feniuk, W., Fozard, J.R., Humphrey, P.P.A., Middlemiss, D.N., Mylecharane, E.J., Richardson,B.P., & Saxena, P.R. (1986). Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine. Neuropharmacology, 25, 563-576.

Briley, M., Chopin, P., & Moret, C. (1990). Effects of serotonergic lesion on "anxious" behaviour measured in the elevated plus-maze in the rat. Psychopharmacology, 101, 187-189.

Carli, M., Prontera, C., & Samanin, R. (1989). Evidence that central 5-hydroxytryptaminergic neurons are involved in the anxiolytic activity of buspirone. British Journal of Pharmacology, 96, 829-836.

Carli, M. & Samanin, R. (1988). Potential anxiolytic properties of 8-hyroxy-2(di-n-propylamino) tetralin, a selective serotonin 1A receptor agonist. Psychopharmacology, 94, 84-91.

Chase, T.N., Katz, R.I., & Kopin, I.J. (1970). Effects of diazepam on the fate of intracisternally injected serotonin-$C^{14}$. Neuropharmacology, 9, 103-108.

Clarke, A. & File, S.E. (1982). Selective neurotoxin lesions of the lateral septum: Changes in social and aggressive behaviours. Pharmacology Biochemistry & Behaviour, 17, 623-628.

Collinge, J., Pycock, C.J., & Taberner, P.V. (1983). Studies on the interaction between cerebral 5-hydroxytryptamine and -aminobutyric acid in the mode of action of diazepam in the rat. British Journal of Pharmacology, 79, 637-643.

Commissaris, R.L., Lyness, W.H., & Rech, R.H. (1981). The effects of d-lysergic acid diethyylamide (LSD), 2,5-dimethoxy-4-methylamphetamine (DOM), pentobarbital, and methaqualone, on punished responding in control and 5,7-dihydroxytryptamine-treated rats. Pharmacology Biochemistry & Behaviour, 14, 617-623.

Commissaris, R.L. & Rech, R.H. (1982). Interactions of metergoline with diazepam, quipazine, and hallucinogenic drugs on a conflict behaviour in the rat. Psychopharmacology, 76, 282-285.

Consolazione, A. & Cuello, A.C. (1982). CNS serotonin pathways. In N.N. Osbourne (ed) Biology of Serotonergic Transmission, New York: John Wiley & Sons.

Cook, L. & Davidson, A.B. (1973). Effects of behaviorally active drugs in a conflict-punishment procedure in rats. In S. Garratini, E. Mussini, & L.O. Randall (ed's), The Benzodiazepines (pp. 327-354). New York: Raven Press.

Cook, L & Sepinwall, J. (1975). Behavioral analysis of the effects and mechanisms of action of benzodiazepines. In E. Costa & P. Greenguard (ed's), Advances in Biochemical Psychopharmacology (v. 14, pp. 1-28). New York: Raven Press.

Critchley, M.A.E. & Handley, S.L. (1987). 5-$HT_{1A}$ ligand effects in the X-maze anxiety test. British Journal of Pharmacology, 92, 660P.

Csanalosi, I., Schwezer, E., Case, W.G., & Rickels, K. (1987). Gepirone in anxiety: A pilot study. Journal of Clinical Psychopharmacology, 7, 31-33.

Dahlstrom, A & Fuxe, K. (1964). Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brainstem neurons. Acta Physiologica Scandinavica, 62, supp. 232, 1-55.

De Boer, S.F., Slangen, J.L., & Van Der Gugten. (1990). Plasma catecholamine and corticosterone levels during active and passive shock-prod avoidance behavior in rats: Effects of chlordiazepoxide. Physiology & Behavior, 47, 1089-1098.

Dunn, R.W., Corbett, R., & Fielding, S. (1989). Effects of the 5-$HT_{1A}$ receptor agonist and NMDA receptor antagonists in the social interaction test and the elevated plus-maze. European Journal of Pharmacology, 169, 1-10.

Engel, J.A., Hjorth, S., Svensson, K., Carlsson, A., & Liljequist, S. (1984). Anticonflict effect of the putative serotonin receptor agonist 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT). European Journal of Pharmacology, 105, 365-368.

Fibiger, H.C. & Campbell, B.A. (1971). The effects of para-chlorophenylalanine on spontaneous locomotor activity In the rat. Neuropharmacology, 10, 25-32.

File, S.E. (1987). The contribution of behavioral studies to the neuropharmacology of anxiety. Neuropharmacology, 26, 877-886.

Fletcher, P.J. & Davies, M. (1990). Dorsal raphe microinjections of 5-HT agonists induces feeding in rats. European Journal of Pharmacology, 184, 265-271.

Gallager, D.W. (1978). Benzodiazepines: Potentiation of a GABA inhibitory response in the dorsal raphe nucleus. European Journal of Pharmacology, 49, 133-143.

Gardner, C.R. (1986). Recent developments in 5HT-related pharmacology of animal models of anxiety. Pharmacology Biochemistry & Behaviour, 24, 1479-1485.

Geller, I. & Blum, K. (1970). The effects of 5-HTP on para-chlorophenylalanine (p-CPA) attenuation of "conflict" behaviour. European Journal of Pharmacology, 9, 319-324.

Geller, I., Hartmann, R.J., Croy, D.J., & Haber, B. (1974). Attenuation of conflict behaviour with cinanserin, a serotonin antagonist: Reversal of the effects with 5-hydroxytryptophan and -methyltryptamine. Research Communications in Chemical Pathology and Pharmacology, 7, 165-174.

Geller, I. & Seifter, J. (1960). The effects of meprobamate, barbiturates, d-amphetamine & promazine in experimentally induced conflict in the rat. Psychopharmacologia, 1, 482-492.

Goa, K.L. & Ward, A. (1986). Buspirone: A preliminary review of its pharmacological properties and therapeutic efficacy as an anxiolytic. Drugs, 32, 114-129.

Gray, D.S., Terlecki, L.I., Treit, D., & Pinel, J.P.J. (1981). Effects of septal lesions on conditioned defensive burying. Physiology & Behaviour, 27, 1051-1056.

Gray, J.A. (1982). The role of the ascending projections to the septo-hippocampal system. The Neuropsychology of Anxiety: An Enquiry into the Functions of the Septo-Hippocampal System. (chap. 11) New York: Oxford University Press.

Green, S. & Hodges, H. (1986). Differential effects of dorsal raphe lesions and intraraphe GABA and benzodiaxepines on conflict behaviour in rats. Behavioral and Neural Biology, 46, 13-29.

Green, S.E., Hodges, H.M., & Summerfield, A. (1983). Evidence for the involvement of brain GABA & serotonin systems in the anti-conflict effects of chlordiazepoxide in rats. British Journal of Pharmacology,79, 265P.

Graeff, F.G. (1974). Tryptamine antagonists and punished behaviour. The Journal of Pharmacology and Experimental Therapeutics, 189, 344-350.

Graeff, F.G. & Schoenfeld, R.I. (1970). Tryptaminergic mechanisms in punished and non-punished behaviour. The Journal of Pharmacology and Experimental Therapeutics, 173, 277-283.

Haefely, W.E. (1989). Pharmacology of the benzodiazepine receptor. European Archives of Psychiatry and Neurological Sciences, 238, 294-301.

Hamon, M., Collin, E., Chantrel, D., Daval, G., Verge, D., Bourgoin, S., & Cesselin, F. (1990). Serotonin receptors and the modulation of pain. In J.-M. Besson, ed. Serotonin and Pain. New York: Elsevier Science Publishers.

Hartmann, R.J. & Geller, I. (1971). p-Chlorophenylalanine effects on a conditioned emotional response in rats. Life Sciences, 10, 927-933.

Higgens, G.A., Bradbury, A.J., Jones, B.J., & Oakley, N.R. (1988). Behavioral and biochemical consequences following activation of 5-$HT_1$-like and GABA receptors in the dorsal raphe nucleus of the rat.

Neuropharmacology, 96, 829-836.

Higgens, G.A., Jones, B.J., & Oakley, N.R. (1987). Compounds selective for the 5-$HT_{1A}$ receptors have anxiolytic effects when injected into the dorsal raphe nucleus of the rat. British Journal of Pharmacology, 92, 658P.

Higley, J.D., Suomi, S.J., & Linniola, M. (1990). Developmental influences on the serotonergic system and timidity in the nonhuman primate. In E.F. Coccaro & D.L. Murphy (ed.s) Serotonin in Major Psychiatric Disorders. (chap. 2) Washington, D.C.: American Psychiatric Press.

Hillegaart, V. (1991). Functional topography of brain serotonergic pathways in the rat. Acta Physiologica Scandinavica, 142, Supp. 598, 1-54.

Hole, K & Lorens, S.A. (1975). Response to electric shock in rats: Effects of selective midbrain raphe lesions. Pharmacology Biochemistry & Behaviour, 3, 95-102.

Hommer, D.W., Skolnick, P., & Paul, S.M. (1987). The benzodiazepine/GABA receptor complex and anxiety. In H.Y. Meltzer (ed) Psychopharmacology: The Third Generation of Progress. (chap. 96) New York: Raven Press.

Iversen, S.D. (1984). 5-HT and anxiety. Neuropharmacology, 23, 1553-1560.

Jacobs, B.L. & Cohen, A. (1976). Differential behavioral effects of lesions of the median or dorsal raphe nuclei in rats: Open field and pain-elicited aggression. Journal of Comparative and Physiological Psychology, 90, 102-108.

Jacobs, B.L., Trimbach, C., Eubanks, E.E., & Trulson, M. (1975). Hippocampal mediation of raphe lesion- and PCPA-induced hyperactivity in the rat. Brain Research, 94, 256-261.

Jacobs, B.L., Wise, W.D., & Taylor, K.M. (1974). Differential behavioral and neurochemical effects following lesions of the dorsal or median raphe nuclei in rats. Brain Research, 79, 353-361.

Jequier, E., Lovenberg, W., & Sjoerdsma, A. (1967). Tryptophan hydroxylase inhibition: The mechanism by which p-chlorophenylalanine depletes rat brain serotonin. Molecular Pharmacology, 3, 274-278.

Ketelaars, C.E.J. & Bruinvels, J. (1989). The anticonflict effect of cyproheptadine is not mediated by its 5-hydroxytryptamine antagonistic property. Life Sciences, 44, 1743-1749.

Kilts, C.D., Commissaris, R.L., Cordon, J.J., & Rech, R.H. (1982). Lack of central 5-hydroxytryptamine influence on the anticonflict activity of diazepam. Psychopharmacology, 78, 156-164.

Kilts, C.D., Commissaris, R.L., & Rech, R.H., (1981). Comparison of anti-conflict drugs in three experimental models of anxiety. Psychopharmacology, 74, 290-296.

Kiser, R.S., Brown, C.A., Sanghera, M.J., & German, D.C. (1980). Dorsal raphe nucleus stimulation reduces centrally-elicited fearlike behaviour. Brain Research, 191, 265-272.

Koe, B.K. & Weissman, A. (1966). p-Chlorophenylalanine: A specific depletor of brain serotonin. The Journal of Pharmacology and Experimental Therapeutics, 154, 499-516.

Korte, S,M. & Bohus,B. (1990). The effects of ipsapirone on behavioral and cardiac responses in the shock-probe/ defensive burying test in male rats. European Journal of Pharmacology, 181, 307-310.

Kostowski, W., Giacalone, E., Garattini, S., & Valzelli, L. (1968). Studies on behavioral and biochemical changes in rats after lesions of the midbrain raphe. European Journal of Pharmacology, 4, 371-376.

Kostowski, W., Plaznik, A., & Stefanski, R. (1989). Intra-hippocampal buspirone in animal models of anxiety. European Journal of Pharmacology, 168, 393-396.

Lal, H. & Emmet-Oglesby, M.W. (1983). Behavioral analogues of anxiety. Neuropharmacology, 22, 1423-1441.

Leysen, J.E. (1985). Characterization of serotonin receptor binding sites. In A.R. Green (ed.), Neuropharmacology of Serotonin. New York: Oxford University Press.

Lister, R.G. (1990). Ethologically-based animal models of anxiety disorders. Pharmacological Therapeutics, 46, 321-340.

Lorens, S.A. & Guldberg, H.C. (1974). Regional 5-hydroxytryptamine following selective midbrain raphe lesions in the rat. Brain Research, 78, 45-56.

Marsden, C.A. & Curzon, G. (1976). Studies on the behavioral effects of tryptophan and p-chlorophenylalanine. Neuropharmacology, 15, 165-171.

Marsden, C.A. & Curzon, G. (1977). Effects of p-chlorophenylalanine and -methyltryptophan on behaviour and brain 5-hydroxyindoles. Neuropharmacology, 16, 489-494.

Meert, T.F. & Colpaert, F.C. (1986) The shock probe conflict procedure. A new assay responsive to benzodiazepines, barbiturates, and related compounds. Psychopharmacology, 88, 445-450.

Molliver, M.E. (1987). Serotonergic neuronal systems: What their anatomic organization tells us about function. Journal of Clinical Psychopharmacology, 7, 3S-23S.

Moret, C. (1985). Pharmacology of the serotonin autoreceptor. In A.R. Green (ed.), Neuropharmacology of Serotonin. New York: Oxford University Press.

Moser, P.C. (1989). An evaluation of the elevated plus-maze test using the novel anxiolytic buspirone. Psychopharmacology, 99, 48-53.

Moser, P.C., Tricklebank, M.D., Middlemiss, D.N., Mir, A.K., Hibert, M.S., & Fozard, J.R. (1990). Characterisation of MDL 73005EF as a 5-$HT_{1A}$ selective ligand and its effects in animal models of anxiety: Comparison with buspirone, 8-OH-DPAT and diazepam. British Journal of Pharmacology, 99, 343-349.

O'Hare, E., Tierney, K.J., & Shephard, R.A. (1991). Cyclic-ratio schedule analysis of a serotonin agonist and depletor on consummatory behaviour. Physiology & Behaviour, 49, 331-334.

Papez, J.W. (1937). A proposed mechanism of emotion. Archives of Neurology and Psychiatry, 38, 725-744.

Parent, A., Descarries, L., & Beaudet, A. (1981). Organization of ascending serotonin systems in the adult rat brain. A radioaoutographic study after intraventricular administration of [3H]5-hydroxytryptamine. Neuroscience, 6, 115-138.

Paxinos, G. & Watson, C. (1986). The Rat brain in Stereotaxic Coordinates (second edition). New York: Academic Press.

Pellow, S., Chopin, P., File, S.E., & Briley, M. (1985). Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. Journal of Neuroscience Methods, 14, 149-167.

Pesold, C. & Treit, D. (1992). Excitotoxic lesions of the septum produce anxiolytic effects in the elevated plus-maze and the shock-probe burying tests. Physiology and Behavior, in press.

Petersen, E.N. & Lassen, J.B. (1981). A water lick conflict paradigm using drug experienced rats. Psychopharmacology, 75, 236-239.

Pinel, J.P.J. & Treit, D. (1978). Burying as a defensive response in rats. Journal of Comparative and Physiological Psychology, 92, 708-712.

Prieto-Gomez, B., Dafny, N., & Reyez-Vazquez, C. (1989). Dorsal raphe stimulation, 5-HT and morphine microiontophoresis effects on noxious and nonnoxious identified neurons in the medial thalamus in the rat. Brain Research Bulletin, 22, 937-943.

Rapport, M.M., Green, A.A., & Page, I.H. (1948). Serum vasoconstrictor (serotonin) IV. Isolation and characterization. The Journal of Biological Chemistry, 176, 1243-1251.

Robichaud, R.C. & Sledge, K.L. (1969). The effects of p-chlorophenylalanine on experimentally induced conflict in the rat. Life Sciences, 8, 965-969.

Rohmer, J-G., Di Scala, G., & Sandner, G. (1990). Behavioral analysis of the effects of benzodiazepine receptor ligands in the conditioned burying paradigm. Behavioural Brain Research, 38, 45-54.

Rosen, A.J. & Freedman, P.E. (1974). The effects of p-chlorophenylalanine on instrumental conditioning in the rat. Neuropharmacology, 13, 585-590.

Sainati, S.M. & Lorens, S.A. (1982). Intra-raphe muscimol induced hyperactivity depends on ascending serotonin projections. Pharmacology Biochemistry & Behaviour, 17, 973-986.

Schoenfeld, R.I. (1976) Lysergic acid diethylamide- and mescaline-induced attenuation of the effect of punishment in the rat. Science, 192, 801-803.

Segal, M. & Landis, S.C. (1974). Afferents to the septal area of the rat studied with the method of retrograde axonal transport of horseradish peroxidase. Brain Research, 82, 263-268.

Seiden, L.S. & Dykstra, L.A. (1977). Serotonin and behaviour. in Psychopharmacology: A Biochemical and

Behavioral Approach (pp 94-105) New York: Van Nostrand Reinhold Company.

Seligman, M.E.P. (1971). Phobias and preparedness. Behaviour Therapy, 2, 307-320.

Sepinwall, J. & Cook, L. (1975). Behavioral pharmacology of antianxiety drugs. In L.L. Iversen, S.D. Iversen, & S.H. Snyder (ed's), Handbook of psychopharmacology (v. 13, pp. 345-393). New York: Plenium Press.

Shephard, R.A., Buxton, D.A., & Broadhurst, P.L. (1982). Drug interactions do not support reduction of serotonin turnover as the mechanism of action of benzodiazepines. Neuropharmacology, 21, 1027-1032.

Soderpalm, B. & Engel, J.A. (1989). Does the PCPA induced anticonflict effect involve activation of the $GABA_A$/ benzodiazepine chloride ionophore receptor complex? Journal of Neural Transmission, 76, 145-153.

Soderpalm, B. & Engel, J.A. (1990). Serotonergic involvement in conflict behaviour. European Journal of Pharmacology, 1, 7-13.

Soderpalm, B. & Engel, J.A. (1991). Involvement of the $GABA_A$/benzodiazepine chloride ionophore receptor complex in the 5,7-DHT induced anticonflict effect. Life Sciences, 49, 139-153.

Soderpalm, B., Hjorth, S., & Engel, J.A. (1989). Effects of $5\text{-}HT_{1A}$ receptor agonists and L-5-HTP in Montgomery's conflict test. Pharmacology Biochemistry & Behaviour, 32, 259-265.

Solomon, P.R., Kiney, C.A., & Scott, D.R. (1978). Disruption of latent inhibition following systematic administration of p-chlorophenylalanine (PCPA). Physiology & Behaviour, 20, 265-271.

Soubrie, P. (1986). Reconciling the role of central serotonin neurons in human and animal behaviour. The

Behavioral and Brain Sciences, 9, 319-364.

Srebro, B & Lorens, S.A. (1975). Behavioral effects of selective midbrain raphe lesions in the rat. Brain Research, 89, 303-325.

Stevens, D.A., Fechter, L.D., & Resnick, O. (1969). The effects of p-chlorophenylalanine, a depletor of brain serotonin, on behaviour: II. Retardation of passive avoidance learning. Life Sciences, 8, 379-385.

Stein, L., Wise, D., & Belluzzi, J.D. (1975). Effects of benzodiazepines on central serotonergic mechanisms. In E. Costa & P. Greengard (ed's), Advances in biochemical psychopharmacology (v. 14, pp. 29-44). New York: Raven Press.

Stein, L., Wise, C.D., & Berger, B.D. (1973). Antianxiety action of benzodiazepines: Decrease in the activity of serotonin neurons in the punishment system. In S. Garrattini, E. Mussini, & L.O. Randall (ed's), The Benzodiazepines (pp. 229-326). New York: Raven Press.

Taylor, D.P., Eison, M.S., Riblet, L.A., & Vandermaelen, C.P. (1985). Pharmacological and clinical effects of buspirone. Pharmacology Biochemistry & Behaviour, 23, 687-694.

Thiebot, M-H., Hamon, M., & Soubrie, P. (1982). Attenuation of induced-anxiety in rats by chlordiazepoxide: Role of raphe dorsalis benzodiazepine binding sites and serotonergic neurons. Neuroscience, 7, 2287-2294.

Thiebot, M-H., Hamon, M., & Soubrie, P. (1983). The involvement of nigral serotonin in the control of punishment-induced behavioral inhibition in rats. Pharmacology Biochemistry & Behaviour, 19, 225-229.

Thiebot, M-H., Soubrie, P., Hamon, M., & Simon, P. (1984). Evidence against the involvement of serotonergic neurons in the anti-punishment activity of diazepam in the rat. Psychopharmacology, 82, 355-359.

Treit, D. (1985a). Animal models for the study of anti-anxiety agents: A review. Neuroscience & Biobehavioral Reviews, 9, 203-222.

Treit, D. (1985b). The inhibitory effect of diazepam on defensive burying: Anxiolytic vs. analgesic effects. Pharmacology Biochemistry & Behaviour, 22, 47-52.

Treit, D. (1987). Ro 15-1788, CGS 8216, picrotoxin, and pentylenetetrazol: Do they antagonize anxiolytic drug effects through an anxiogenic action? Brain Research Bulletin, 19, 401-405.

Treit, D. (1990). A comparison of anxiolytic and nonanxiolytic agents in the shock-probe/burying test for anxiolytics. Pharmacology Biochemistry & Behaviour, 36, 203-205.

Triet, D & Fundytus, M. (1988). A comparison of buspirone and chlordiazepoxide in the shock-probe/burying test for anxiolytics. Pharmacology Biochemistry & Behaviour, 31, 1071-1075.

Treit, D & Menard, J. (1991). Personal communication.

Treit, D & Pesold, C. (1990). Septal lesions inhibit fear reactions in two animal models of anxiolytic drug action. Physiology & Behaviour, 47, 365-371.

Treit, D., Pinel, J.P.J., & Fibiger, H.C. (1981). Conditioned Defensive burying: A new paradigm for the study of anxiolytic agents. Pharmacology Biochemistry & Behaviour, 15, 619-626.

Tsuda, A., Ida, Y., Nishimura, H., & Tanaka, M. (1989). Anxiogenic effects of -CCE as measured in two different conditioning paradigms. Psychobiology, 17, 202-206.

Tsuda, A., Ida, Y., & Tanaka, M. (1988). The contrasting effects of diazepam and yohimbine on conditioned defensive burying in rats. Psychobiology, 16, 213-217.

Twarog, B.M. & Page, I.H. (1953). Serotonin content of some mammalian tissues and urine and a method for its determination. Journal of Physiology, 175, 157-161.

Tye, N.C., Everitt, B.J., & Iversen, S.D. (1977). 5-Hydroxytryptamine and punishment. Nature, 268, 741-743.

Tye, N.C., Iversen, S.D., & Green, A.R. (1979). The effects of benzodiazepines and serotonergic manipulations on punished responding. Neuropharmacology, 18, 689-695.

VanderMaelen, C.P., Matheson, G.K., Wilderman, R.C., & Pattersen, L.A. (1986). Inhibition of serotonergic dorsal raphe neurons by systemic and iontophoretic administration of buspirone, a non-benzodiazepine anxiolytic drug. European Journal of Pharmacology, 129, 123-130.

Vogel, J.R., Beer, B., & Clody, D.E. (1971). A simple and reliable conflict procedure for testing anti-anxiety agents. Psychopharmacology, 21, 1-7.

Walletschek, H. & Raab, A. (1982). Spontaneous activity of dorsal raphe neurons during defensive and offensive encounters in the tree-shrew. Physiology & Behaviour, 28, 697-7055.

Wang, S.S. & Peroutka, S.J. (1988). Historical Perspectives. In E. Sanders-Bush (ed) The Serotonin Receptors. New Jersey: The Humana Press.

Wing. L.L. & Wirtshafter, D. (1982). Impaired DRL performance with electrolytic median raphe lesions. Society for Neuroscience Abstracts. 12th annual meeting, Minneapolis.

Winter, J.C. (1972). Comparison of chlordiazepoxide, methylsergide, and cinanserin, as modifiers of punished behaviour and as antagonists of n-n-dimethyltryptamine. Archives of International Pharmacodynamics, 197, 147-159.

Wirtshafter, D., Montana, W., & Asin, K.E. (1983). Stimulus discriminability and learning following median raphe

lesions. Society for Neuroscience Abstracts. 13th annual meeting, Boston.

Wise, C.D., Berger, B.D., & Stein, L. (1972). Benzodiazepines: Anxiety-reducing activity by reduction of serotonin turnover in the brain. Science, 177, 180-183.