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# The Role of the Dorsal Hippocampal Serotonergic and Cholinergic Systems in the Modulation of Anxiety

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FILE, S. E., P. J. KENNY AND S. CHEETA. *The role of the dorsal hippocampal serotonergic and cholinergic systems in the modulation of anxiety.* PHARMACOL BIOCHEM BEHAV **66**(1) 65–72, 2000.—A review of the literature suggests that the dorsal hippocampal serotonergic system, and, in particular, the postsynaptic 5-$HT_{1A}$ receptor, mediates an anxiogenic response, whereas endogenous dorsal hippocampal cholinergic tone mediates an anxiolytic response. Accordingly, it has been shown that direct dorsal hippocampal administration of the 5-$HT_{1A}$ receptor agonist, 8-OH-DPAT, the nicotinic receptor antagonist, mecamylamine, and the $M_1$ muscarinic receptor antagonist, pirenzepine, all have anxiogenic effects in rats tested in the social interaction test. It is therefore surprising that nicotine also has an anxiogenic effect in this test following dorsal hippocampal administration. However, the anxiogenic effects of mecamylamine and nicotine in the dorsal hippocampus are blocked by coadministration of the 5-$HT_{1A}$ receptor antagonist, WAY 100635, suggesting that both of these compounds act by enhancing hippocampal serotonergic transmission, thereby stimulating postsynaptic 5-$HT_{1A}$ receptors. This conclusion is supported by the observation that both nicotine and mecamylamine stimulate basal [$^3$H]-5-HT release from dorsal hippocampal slices. A possible mechanism by which nicotinic receptor ligands modulate hippocampal 5-HT release is discussed, and it is proposed that the dorsal hippocampal serotonergic and cholinergic systems are tightly coupled and function antagonistically in the modulation of anxiety, as measured in the social interaction test. These systems are relatively unimportant in controlling behaviour on trial 1 in the plus-maze. On trial 2 in the elevated plus-maze, a model of specific phobia, the endogenous cholinergic system, nicotine, and the $M_1$ receptor agonist, McN-A-343, all mediate an anxiolytic effect, whereas stimulation of 5-$HT_{1A}$ receptors mediates an anxiogenic effect. It is proposed that the hippocampus may predominantly control the avoidance components of phobic anxiety, with other regions, such as the dorsomedial hypothalamus, controlling the escape components. © 2000 Elsevier Science Inc.

Nicotine Mecamylamine Pirenzepine McN-A-343 $M_1$ muscarinic receptor 8-OH-DPAT 5-$HT_{1A}$ receptor Glycine Dorsal hippocampus Anxiety Rat

THERE is considerable evidence that nicotine can modulate anxiety, but it is also clear that it does not universally reduce anxiety in the way that is observed with the benzodiazepines. Several factors are crucial in determining not only whether effects of nicotine are observed but the direction of nicotine's effects on anxiety. Within a single test of anxiety, the social interaction test, the effects of IP administration of (−)-nicotine have been shown to be dose dependent, with low doses having an anxiolytic, and high doses an anxiogenic, effect (22). The same low dose (0.1 mg/kg) has been found to have different effects at different times after injection, with an anxiogenic effect being observed at 5 min, followed by an anxiolytic effect at 30 min, and a later anxiogenic effect at 60 min (37). In the elevated plus-maze test of anxiety, Ouagazzal et al. (50) found no effects of (−)-nicotine at doses of 0.001, 0.005, 0.01, 0.05, and 0.1 mg/kg, but anxiogenic effects at 0.5 and 1 mg/kg; Balfour et al. (3) found no effect of the racemate at 0.4 mg/kg, whereas Brioni et al. (5) found an anxiolytic effect at a single dose of (−)-nicotine 0.3 mg/kg. It is possible that the different effects observed by Brioni et al. (5) and Ouagazzal et al. (50) were due to strain effects and/or the different baseline levels of anxiety in the two experiments. The scores were much

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higher in the Ouagazzal et al. (50) study, indicating a lower level of anxiety. The baseline level of anxiety, as generated by the different test conditions, is also an important factor in determining the effects of nicotine in the social interaction test. Thus, in the condition generating the least anxiety (a familiar test arena, lit by low light-LF) nicotine is without effect, as it is in the condition of highest anxiety (an unfamiliar arena, lit by high light-HU); it is in the two conditions generating moderate levels of anxiety (unfamiliar arena lit by low light-LU, and familiar arena, lit by high light-HF) that the bidirectional effects of systemically administered nicotine can be observed (22).

## ROLE OF THE DORSAL HIPPOCAMPUS IN ANIMAL TESTS OF ANXIETY

The dorsal hippocampus is one brain region that plays an important role in anxiety (33), and both benzodiazepines and drugs acting on the 5-HT system have been found to have effects in animal tests of anxiety after direct administration into the dorsal hippocampus. However, it is also clear that this brain region is more important for controlling behaviour in some animal tests than in others.

### *Social Interaction*

The social interaction test provides a good model of generalised anxiety disorder (GAD), and the dorsal hippocampus plays an important role in controlling behaviour in this test. After direct administration to the dorsal hippocampus, midazolam (1 and 2 μg) is anxiolytic in this test (30), whereas the 5-$HT_{1A}$ receptor agonist, 8-OH-DPAT (100 ng) and the 5-$HT_{2C}$ receptor agonist, mCPP (500 ng) have anxiogenic effects (2,17,67). In conditions of low anxiety, both the nicotinic receptor antagonist, mecamylamine, and the muscarinic cholinergic receptor antagonists, pirenzepine and scopolamine, have anxiogenic effects (18,58). These results suggest that the endogenous cholinergic tone in the dorsal hippocampus plays a modulatory role to reduce anxiety. It was therefore surprising that, under conditions of moderate anxiety, injection of nicotine into this brain region has anxiogenic effects (22,50).

### *Vogel Punished Drinking*

Factor analysis has shown that the type of anxiety detected in the Vogel test is distinct from that measured in the social interaction test (12), and hence, different brain regions and neurotransmitters might be involved. In the Vogel punished-drinking test, the hippocampus again seems to play a role, although the doses of drugs administered to the dorsal hippocampus are extremely high, which raises the question of pharmacological and neuroanatomical specificity. Thus, the benzodiazepines, midazolam (10 and 20 μg) and diazepam (40 μg), the 5-$HT_{1A}$ partial agonists, 8-OH-DPAT (1), buspirone, ipsapirone (1 and 3 μg), gepirone (10 and 30 μg), and tandospirone (30 and 60 μg), and the 5-$HT_3$ receptor antagonist, ondansetron (1 and 2 μg) have all been reported to have anxiolytic effects (39,53,59). There is one report of an anxiogenic effect following 5 μg buspirone (59). The bulk of the evidence, however, suggests that the hippocampal 5-$HT_{1A}$ receptors play an opposite role in modulating anxiety in the Vogel punished-drinking test and in the social interaction test. This could arise if the two tests generate quite distinct states of anxiety, with distinct underlying neurobiological mechanisms. This could account for the much higher doses of benzodiazepines needed to modify behaviour in the Vogel test, compared with the social interaction test, because not all anxiety disorders are equally well treated by benzodiazepines. Additionally, or alternatively, the dorsal hippocampus may play a less important role in the state generated by the Vogel test than it does in the state generated by the social interaction test.

However, there is an important, alternative explanation. Using the USV test (ultrasonic vocalisations induced by foot shock) Jolas et al. (38) found anxiolytic effects after administration of 8-OH-DPAT (1–10 μg) into the dorsal hippocampus. These doses are higher than those needed to produce an anxiolytic effect after IV administration, which strongly suggests that the hippocampus is not the main anatomical target of anxiolytic action, and furthermore, that this area may mediate an anxiogenic effect. This anxiogenic effect would act to reduce the anxiolytic effect mediated in other areas. Jolas et al. (38) further showed that the anxiolytic effect of these high doses of 8-OH-DPAT was not due to a hippocampal site of action, because the effect remained even when administered to a dorsal hippocampus that had been lesioned with ibotenic acid, thus destroying the 5-$HT_{1A}$ receptors in this region. They showed that these high doses of 8-OH-DPAT had diffused back to the dorsal raphé nucleus, and that it was probably an action in this brain region that accounted for both the observed anxiolytic action and the reduction in raphé firing rate, and hence, the release of 5-HT in limbic projection areas. Certainly, it has been shown that direct administration of 8-OH-DPAT into the dorsal raphé nucleus has anxiolytic effects in the 20–50-ng range in the social interaction and USV tests (34,35,57) and in the 200–500-ng range in the Vogel test (34). Eison et al. (8) found that 5,7 DHT lesions of the 5-HT neurones blocked the anxiolytic effects of buspirone and gepirone in the Vogel test, thus indicating a presynaptic site of action.

In the Vogel test, there is a fivefold difference in the effective anxiolytic doses of drugs stimulating 5-$HT_{1A}$ receptors in the raphé nuclei and the dorsal hippocampus. Whilst this may be explained by diffusion of the drug from its site of injection to a more distant site of action, it could also be explained by the fact that the compounds act as full agonists at the presynaptic sites, but as partial agonists at postsynaptic sites. For example, in the dorsal hippocampus, 8-OH-DPAT has only about one-quarter the intrinsic activity of 5-HT to inhibit neuronal activity (1). Whether an agonist or antagonist action is seen following administration of a partial agonist will depend on the level of 5-HT tone. Thus, in rats depleted of 5-HT by the administration of parachlorophenylalanine, 8-OH-DPAT had clear anxiogenic effects in the Vogel test (9), probably due to a postsynaptic site of action. In the conditioned suppression of drinking test, the effect of 8-OH-DPAT was found to be dependent on the arousal level of the animal; thus, it was anxiolytic in naive rats and anxiogenic in habituated rats (7). Habituation to handling has been shown to reduce the endogenous 5-HT tone in the hippocampus (16), and thus it is likely that the anxiogenic effect of 8-OH-DPAT is observed in test conditions that engender low levels of hippocampal 5-HT.

The endogenous 5-HT tone will also determine the pre- and postsynaptic balance of effects, with high hippocampal 5-HT tone favouring a presynaptic action and a low 5-HT tone a postsynaptic action. A high hippocampal 5-HT tone would also favour detection of a 5-$HT_{1A}$ antagonist action from a partial agonist. The Vogel punished-drinking test, using 0.4-mA shock level, gives rise to plasma corticosterone concentrations four times higher than the social interaction test and three times higher than the elevated plus-maze (21). Matsuo et al. (47) found increased dorsal hippocampal 5-HT

release during punished drinking. Thus, the much greater level of stress caused by the Vogel test is accompanied by high 5-HT concentrations in the dorsal hippocampus, and thus the anxiolytic effects observed following administration of high doses of 8-OH-DPAT and buspirone could be due due to their 5-$HT_{1A}$ receptor antagonist properties. This would be supported by the finding that pindolol, a 5-$HT_{1A}$ receptor antagonist, was anxiolytic at a low dose (300 ng) when administered to the dorsal hippocampus (52). Puzzlingly, another 5-$HT_{1A}$ receptor antagonist, WAY 100635, was silent. Przegalinski et al. (53) found that the effect of intrahippocampal administration of ipsapirone was reversed by coadministration of NAN-190, which would seem to argue against a 5-$HT_{1A}$ receptor antagonist action underlying the anxiolytic effect of a high doses of ipsapirone. However, both compounds also have actions at 5-$HT_7$ receptors (45,69), and thus the pharmacological specificity of their actions cannot be certain. In conclusion, whilst there is agreement between the social interaction and Vogel tests that 5-$HT_{1A}$ receptor agonist action in the raphé nuclei gives rise to anxiolytic effects, the source of the anxiolytic action following administration of high doses of partial agonists to the dorsal hippocampus remains unclear. A spread of the drugs back to the raphé is one possibility, but a 5-$HT_{1A}$ receptor antagonist and/or a 5-$HT_7$ receptor agonist action are alternatives. Of course, it is possible that the answer will differ for different drugs that vary in their intrinsic activity at pre- and postsynaptic 5-$HT_{1A}$ receptors and at 5-$HT_7$ receptors.

*Elevated Plus-Maze*

*Trial 1—naive rats.* File (12) showed that the elevated plus-maze detects a quite different type of anxiety from either the social interaction test or the Vogel punished-drinking test, and Graeff et al. (31,32) has suggested that the plus-maze best models aspects of panic disorder. There is strong evidence for important control from midbrain regions, such as the raphé nuclei and the periaqueductal grey (14,17,31), but the dorsal hippocampus does not seem to play an important role in controlling behaviour on trial 1 in the plus-maze. Thus, Treit and Menard (62) found that lesions of the dorsal hippocampus were without effect on performance in the plus-maze. File et al. (18) found no effect of nicotinic or muscarinic receptor antagonists administered directly to the dorsal hippocampus, Ouagazzal et al. (50) found no effects of a wide dose range of nicotine, Gonzalez et al. (30) found no effects of midazolam (1 and 2 μg), and no effects of 8-OH-DPAT were found at 1 μg (4) or 50–200 ng (17). However, in test conditions that yielded a very low baseline level of responding (10% time spent on open arms), an anxiolytic effect of R(+)8-OH-DPAT has been found (48). Kostowski et al. (42) also reported an increase in time spent on open arms after buspirone (2.5 μg), but in this experiment the significance arose because the groups differed in the variances of their scores (SEM 7.5 in the control group and 41.5 in the buspirone group). The apparent difference in mean was therefore probably due to an isolated high score. Entries into open arms were not reported, so it is not possible to see whether there was an increase in the other measure of anxiety, and because closed-arm entries were not reported, it is not possible to see whether measures of nonspecific activity were also changed. However, the Menard and Treit finding does suggest that, in certain conditions, the hippocampus can influence plus-maze performance. Prior exposure to stress, for example, restraint stress or brief exposure to cat odour (49,71) decreases the baseline scores in the plus-maze. Thus, the lower scores in the Menard and Treit study (48) could indicate that their rats are more stressed than ours. Several procedural differences could contribute to this. For example, the Menard and Treit rats arrive by air freight, are immediately singly housed, and their behaviour on the plus-maze is scored by an observer in the same room (Menard, personal communication). Ours have a short road journey, have 1 week's group housing and handling before single housing, and are tested alone in a small room and observed from a video monitor in an adjacent area.

One possibility, therefore, is that the hippocampus plays a role in stress-induced decreases in behaviour, regardless of the test situation in which they are detected. Thus, dorsal hippocampal lesions prevented the decrease in locomotor activity induced by FG 7142 (44), and 8-OH-DPAT (5 μg) administration to the dorsal hippocampus reduced stress-induced decreases in locomotor activity (6). Administration of the nonselective 5-$HT_1$ receptor agonist, 5-MeODMT, to the dorsal hippocampus immediately after 2 h of restraint stress was able to reverse the decrease in percent time spent on open arms and percent of open arm entries in the plus-maze that was detected 24 h after the restraint and drug injection (49). Thus, the dorsal hippocampal 5-$HT_{1A}$ receptors seem to play an important part in mediating stress-induced hypoactivity, as detected both by decreased locomotor activity and by changes in the plus-maze. However, this is not necessarily the same as playing a direct role in mediating behaviour in the plus-maze.

There is evidence that testing in the plus-maze increases hippocampal 5-HT release, but unfortunately, the specific role of the dorsal hippocampus has not been assessed. File et al. (23) found increased [$^3$H]-5-HT release and decreased uptake in slices taken across the whole hippocampus and therefore including both dorsal and ventral hippocampus; and, using in vivo microdialysis, Wright et al. (70) and Voigt et al. (66) found increased 5-HT in the ventral hippocampus. However, the increase was the same whether the animals were restricted to the open or closed arms, and the 5-HT release was not related to the level of anxiety displayed in the plus-maze (70).

*Trial 2.* Dramatic changes occur during the first 5-min exposure to the elevated plus-maze. On trial 1, the main source of anxiety is the open nature of the arms (63), whereas by the second 5-min trial it is the elevation of the arms that is the controlling factor (10). The type of anxiety measured on trials 1 and 2 is quite distinct (10,13,15,54), and behaviour on trial 2 is insensitive to benzodiazepines (11,55,56). The lack of sensitivity to benzodiazepines is not due to habituation of anxiety because the scores on trial 2 either remain unchanged (11,51,61) or decrease (4,29,56), indicating enhanced anxiety. Nor is there any habituation from trial 1 to trial 2 of the corticosterone stress response (25,36). Because of the importance of the fear of heights on trial 2 and the insensitivity to benzodiazepines, it has been suggested that trial 2 in the elevated plus-maze may be a good model of simple, or specific, phobias (15,19,20,26). Fear of heights is the most common of specific phobias (28), and benzodiazepines are ineffective against them (46,64).

In contrast to trial 1, there is clear evidence that the dorsal hippocampus plays a role in modulating behaviour on trial 2. This is possibly because of the crucial role played by learning in establishing the fear of heights on trial 2 (10,16). 8-OH-DPAT (100 ng) had a significant anxiogenic effect on trial 2 in the plus-maze after direct administration to the dorsal hippocampus, and this effect was antagonised by the specific

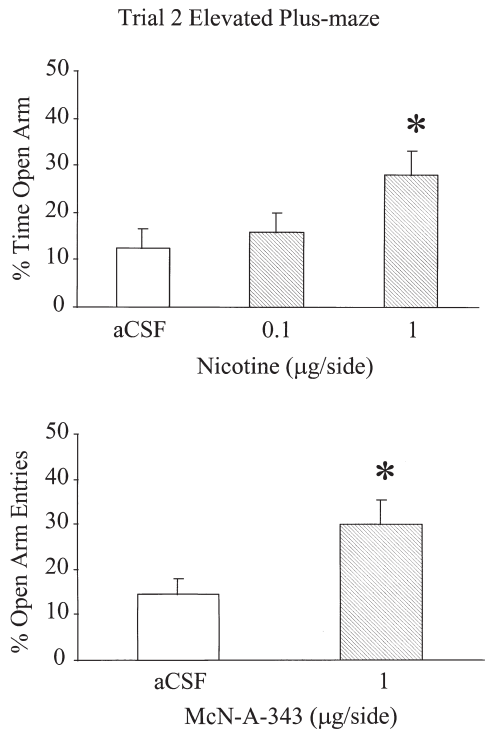


FIG. 1. Uppper panel: mean (±SEM) percent time spent on open arms by rats on trial 2 in the elevated plus-maze after bilateral dorsal hippocampal injections of artifical CSF (aCSF) or nicotine (0.1 and 1 μg/side). *$p < 0.05$ compared with aCSF control group. [Reproduced with permission from Ouagazzal et al. (50).] Lower panel: mean (±SEM) percnet open arms entries by rats on trial 2 in the elevated plus-maze after bilateral dorsal hippocampal injections of artifical CSF (aCSF) or McN-A-343 (1 μg/side). *$p < 0.05$ compared with aCSF control group.

5-$HT_{1A}$ receptor antagonist, WAY 100635 (17). The nicotinic receptor antagonist, mecamylamine, and the $M_1$ muscarinic receptor antagonist, pirenzepine, had anxiogenic effects on trial 2 after dorsal hippocampal administration (18), whereas nicotine and the $M_1$ muscarinic receptor agonist, McN-A-343, had anxiolytic effects [(50); Fig. 1].

## CHOLINERGIC AND SEROTONERGIC RELEASE IN THE DORSAL HIPPOCAMPUS

Glutamatergic neurones comprise 90% of the neurones in the hippocampus (pyramidal and granule cells), and the remaining 10% of cells are GABAergic interneurones (27), and both glutamate and GABA are involved in synaptic interactions. In addition, the hippocampal neuropil is enriched by noradrenergic, serotonergic, and cholinergic axon terminals, and the release of these neurotransmitters plays a modulatory role. The serotonergic input to the dorsal hippocampus comes mainly from the median raphé nucleus, and these fibres are studded with boutons that form synapses (unlike the small fibres from the dorsal raphé, which form nonsynaptic contacts). The cholinergic input to the dorsal hippocampus comes from the median septum, and acetylcholine (ACh) plays a role in nonsynaptic interactions and can thus influence receptors quite distant from the site of release. Stimulation of presynaptic muscarinic $M_2$ autoreceptors inhibits the release of acetylcholine, whereas stimulation of presynaptic nicotinic autoreceptors stimulates acetylcholine release, through a nicotinic receptor that does not contain the α7 subunit [for review, see (65)]. Serotonergic stimulation of the 5-$HT_{1B}$ heteroreceptors decreases acetylcholine release (65); thus, as the serotonergic tone of the dorsal hippocampus is increased, so the cholinergic tone is decreased. There is also good evidence for a cholinergic modulation of 5-HT release in the hippocampus. Stimulation of the 5-$HT_{1B}$ autoreceptors decreases 5-HT release in the dorsal hippocampus, as does stimulation of muscarinic $M_1$ heteroreceptors located on the serotonergic terminals. However, Lendvai et al. (43) showed that the nicotinic receptor agonists, DMPP and lobeline, stimulate 5-HT release, although the release was not calcium sensitive, suggesting a nonclassical mechanism.

## ENDOGENOUS CHOLINERGIC AND SEROTONERGIC TONE IN DIFFERENT LEVELS OF ANXIETY

As previously stated, the social interaction test provides the opportunity of varying the level of anxiety that is generated by the test conditions. Thus, anxiety is lowest when the rats are tested under low light in an arena with which they are familiar (LF test condition). In this test condition, there is high cholinergic tone in the dorsal hippocampus, as indicated by the anxiogenic effects of mecamylamine and pirenzepine when they are injected directly into this area (18). However, in test conditions that generate higher anxiety, mecamylamine is without effect (22), suggesting that in these test conditions there is relatively little endogenous cholinergic tone. Thus, the endogenous cholinergic tone in the dorsal hippocampus decreases with increases in anxiety.

There is evidence for increasing serotonergic tone in the dorsal hippocampus with increasing anxiety. Following administration of pentylenetetrazole (15 mg/kg), rats had a significant increase in anxiety and increased concentrations of 5-HT and glycine in the dorsal hippocampus, as measured by in vivo microdialysis (see Fig. 2). However, it is not known whether these increases in 5-HT and glycine will arise under all conditions of increased anxiety, and it is possible that some, but not all, of the anxiety tests will generate such changes. Microdialysis experiments have also shown that exposure to the Vogel punished-drinking test increases 5-HT concentrations in the dorsal hippocampus of rats (47). Increases in [$^3$H]-5-HT release from hippocampal slices that include both dorsal and ventral areas has been found in rats exposed to the high light, familiar test condition of the social interaction test (23).

## EVIDENCE THAT THE ANXIOGENIC EFFECTS OF MECAMYLAMINE IN THE SOCIAL INTERACTION TEST ARE MEDIATED BY INCREASED 5-HT RELEASE IN THE DORSAL HIPPOCAMPUS

The anxiogenic effects of mecamylamine when injected into the dorsal hippocampus could be the result of an increase in 5-HT release, caused by antagonising the action of endogenous acetylcholine acting at the nicotinic autoreceptor ($N_A$ in Fig. 3), thereby decreasing the inhibitory influence of the $M_1$ receptor on 5-HT release. To test this hypothesis, the effect of mecamylamine on [$^3$H]-5-HT release from hippocampal slices was investigated. Figure 4 shows that mecamylamine did indeed enhance 5-HT release. Further evidence that this increase in 5-HT

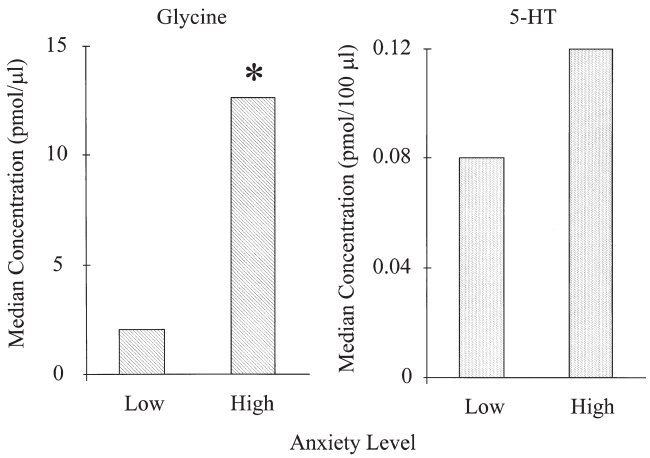


FIG. 2. Median dorsal hippocampal concentrations of glycine (pmol/µl) and 5-HT (pmol/100 µl) in a group of rats with low and high levels of anxiety following injection of pentylenetetrazole (15 mg/kg). *$p < 0.05$ compared with control [see File et al. (24)].

release was mediated by reduced acetylcholine acting at a muscarinic $M_1$ cholinergic heteroreceptor, as shown in Fig. 3, comes from the increased 5-HT release induced by the $M_1$ receptor antagonist, pirenzepine [(41); Fig. 4).

The ability of mecamylamine to increase 5-HT release was antagonised by glycine, acting at a strychnine-insensitive receptor (see Fig. 4). The NMDA receptor, which possesses the strychnine-insensitive glycine receptor at which glycine acts as a coagonist, also has an inhibitory action on hippocampal 5-HT release (60,68). However, glycine alone was without effect on basal 5-HT release, but this may have been because basal 5-HT release was already so low that it was not possible to observe a further reduction.

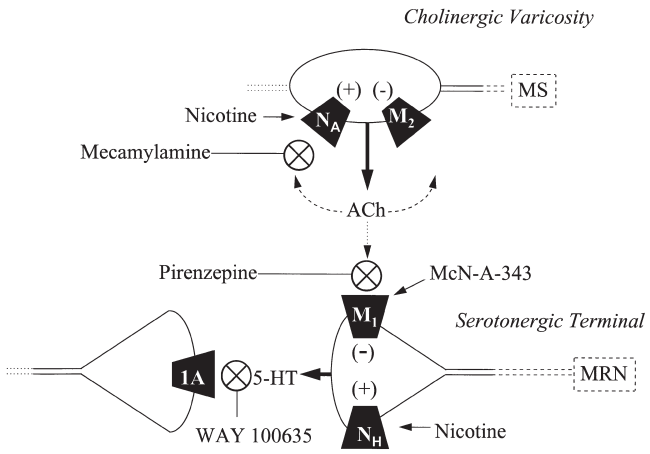


FIG. 3. Proposed model by which nicotinic and $M_1$ muscarinic receptor ligands modulate hippocampal serotonin release. ACh, acetylcholine; 5-HT, serotonin; MS, medial septum; MRN, median raphé nucleus; $M_1$, mucsarinic $M_1$ receptor; $M_2$, muscarinic $M_2$ receptor; 1A, postsynaptic 5-$HT_{1A}$ receptor; NA, nicotinic autoreceptor; NH, nicotinic heteroreceptor; (+), indicates a stimulatory action; (−), indicates an inhibitory action.

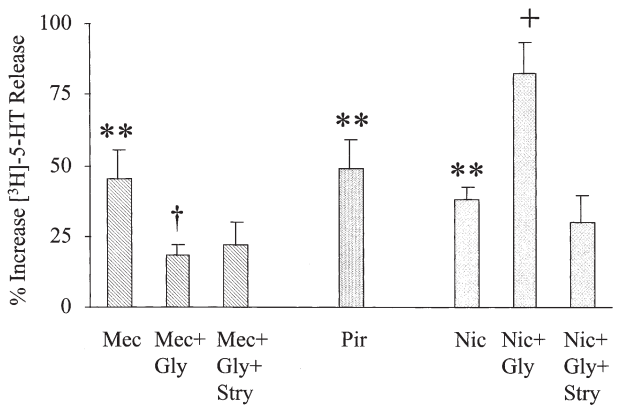


FIG. 4. Left panel: mean (±SEM) percent increase in resting release of [$^3$H]-5-HT from rat dorsal hippocampal slices after mecamylamine (50 mM; Mec), mecamylamine (50 mM) + Glycine (20 mM) (Mec + Gly), and mecamylamine + glycine (20 mM) + strychnine (20 mM) (Mec + Gly + Stry) superfusion. Centre panel: mean (±SEM) percent increase in resting release of [$^3$H]-5-HT from rat dorsal hippocampal slices after pirenzepine (0.05 mM; Pir) superfusion. Right panel: mean (±SEM) percent increase in resting release of [$^3$H]-5-HT from rat dorsal hippocampal slices after nicotine (250 mM; Nic), nicotine (250 mM) + Glycine (20 mM) (Nic + Gly) and nicotine + glycine (20 mM) + strychnine (20 mM) (Nic + Gly + Stry) superfusion. **$p < 0.01$ compared with control, *$p < 0.05$ compared with Mec, $^+p <$ 0.05 compared with Nic.

The anxiogenic effects of mecamylamine were reversed by coadministration of the 5-$HT_{1A}$ receptor antagonist, WAY 100635 (see Fig. 5), thus suggesting that the anxiogenic effects were due to increased 5-HT release, acting at postsynaptic 5-$HT_{1A}$ receptors.

### EVIDENCE THAT THE ANXIOGENIC EFFECTS OF NICOTINE IN SOCIAL INTERACTION ARE DUE TO INCREASED 5-HT RELEASE

The anxiogenic effects of nicotine in the social interaction test were not reversed by coadministration into the dorsal hippocampus of the $M_1$ receptor antagonist, pirenzepine (40). Thus, the effects of nicotine do not seem to be mediated by an increase in cholinergic transmission. However, the anxiogenic effects of nicotine were significantly reversed by coadministration of WAY 100635 [Fig. 5; (40)], suggesting that they were mediated by increased 5-HT release acting at postsynaptic 5-$HT_{1A}$ receptors. Although nicotine has been found to increase 5-HT release in many brain regions, it was less effective at stimulating 5-HT release in the dorsal hippocampus than were other nicotinic receptor agonists (43). It therefore seems that the nicotinic receptor in this brain region might differ in its subunit composition from that found in other regions. Figure 4 shows that nicotine does significantly increase [$^3$H]-5-HT release from dorsal hippocampal slices, but at rather high concentrations. The release of 5-HT from the dorsal hippocampus elicited by nicotinic agonists is calcium independent and TTX insensitive (41,65). We therefore propose that nicotine is acting at an atypical heteroreceptor on 5-HT terminals ($N_H$ in Fig. 3). The ability of nicotine to enhance 5-HT release is potentiated by glycine, acting at a strychine-sensitive receptor, in contrast to that induced by mecamylamine. Glycine alone had no effect on resting release, which could be

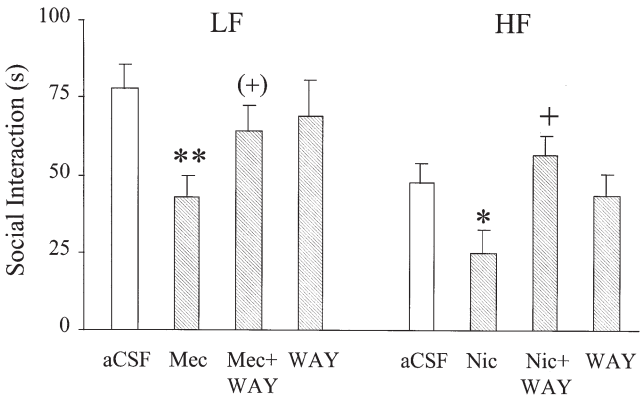


FIG. 5. Left panel: mean (±SEM) time spent in social interaction in the low light-familiar (LF) test condition by rats tested after bilateral dorsal hippocampal injections of artificial CSF (aCSF), mecamylamine (100 ng/side; Mec), mecamylamine (100 ng/side), and WAY 100635 (200 ng/side) (Mec + WAY) or WAY 100635 (200 ng/side; WAY). $^{**}p < 0.01$ compared with aCSF control; $(+)p = 0.07$ compared with Mec, (Kenny, Cheeta, and File, unpublished observation). Right panel: mean (±SEM) time spent in social interaction in the high light-familiar (HF) test condition by rats tested after bilateral dorsal hippocampal injections of aftificial CSF (aCSF), nicotine (8 mg/side; Nic), nicotine (8 mg/side), and WAY 100635 (200 ng/side; Nic + WAY) or WAY 100635 (200 ng/side; WAY). $^{*}p < 0.05$ compared to aCSF control; $^{+}p < 0.05$ compared to Nic [see Kenny et al., [40]).

because glycine only acts in this case to reduce the nicotine-stimulated release of a second neurotransmitter (e.g., noradrenaline), which has an inhibitory action on 5-HT release.

## BALANCE BETWEEN CHOLINERGIC ANXIOLYTIC AND SEROTONERGIC ANXIOGENIC EFFECTS IN DIFFERENT TESTS OF ANXIETY

### *Social Interaction*

There is mutual inhibition between the cholinergic and serotonergic systems, and we propose that, under low levels of anxiety in the social interaction test, as generated in the LF test condition, the endogenous cholinergic tone dominates the endogenous serotonergic tone in the dorsal hippocampus. This endogenous cholinergic tone has an anxiolytic modulatory effect, and hence, anxiogenic effects can easily be observed following local injection of muscarinic or nicotinic receptor antagonists. The low serotonergic tone is accompanied by low concentrations of glycine, and thus the nicotinic stimulation of 5-HT release is not enhanced and is dominated by the powerful inhibition of 5-HT release by endogenous acetylcholine acting at $M_1$ receptors. This powerful inhibition of 5-HT release is revealed by the effects of pirenzepine, which at concentrations of 5 nM could increase 5-HT release (41). This can be contrasted with the need for 100 μM nicotine to increase 5-HT release.

In conditions of moderate anxiety, there is increased 5-HT tone, and this now dominates the low cholinergic tone. Accompanying the increased 5-HT tone is an increase in glycine, which acts to enhance the nicotine-induced increase in 5-HT release. In the HU test conditon, which generates the highest level of anxiety, nicotine may be unable to further enhance 5-HT release, and hence, it remains behaviourally silent. Thus, the dorsal hippocampal serotonergic and cholinergic systems are both biochemically and behaviourally tightly coupled, and appear to have an antagonistic relationship in the modulation of anxiety, as measured in the social interaction test.

### *Trial 1 in the Plus-Maze*

As was reviewed in a previous section, the dorsal hippocampus in general, and in particular, the endogenous cholinergic system, does not seem to play an important role in controlling anxiety on trial 1 in the plus-maze. It may, however, play an indirect role by mediating the effects of stress, that can change baseline performance in the plus-maze.

### *Trial 2 in the Elevated Plus-Maze*

In rats that have previously received a 5-min trial in the plus-maze, the dorsal hippocampus becomes important in modulating behaviour on trial 2. The endogenous dorsal hippocampal cholinergic tone is anxiolytic in this test, with both pirenzepine and mecamylamine having anxiogenic effects. Furthermore, nicotine and McN-A-343 are able to further add to this tone, and themselves exert anxiolytic effects. However, doses of nicotine higher than 1 μg were no longer anxiolytic (50), suggesting that at higher doses some other action of nicotine was counteracting the anxiolytic effect. Phobic behaviour has two distinct components—avoidance of the phobic object, and, when confronted with it, intense anxiety and escape from it (46). It is possible that the dorsal hippocampus is more concerned with the avoidance component, and that other brain regions, such as the dorsomedial hypothalamus (20), are more concerned with the escape components.

The moderate to high cholinergic tone on trial 2 would seem to be accompanied by a fairly low serotonergic tone, as revealed by the anxiogenic effects following direct dorsal hippocampal 8-OH-DPAT (17). Thus, although the anxiolytic effects of hippocampally administered nicotine and McN-A-343 could arise through their ability to further reduce serotonergic tone, this is not necessarily the mechanism and other neurotransmitters could be involved.

In the social interaction test, a high cholinergic tone and low serotonergic tone in the dorsal hippocampus was characteristic of the least anxiety provoking test situation, i.e., LF. It might therefore seem that trial 2 in the elevated plus-maze is one that generates a low level of anxiety. This is certainly not the case, as judged by both behavioural and corticosterone measures. It therefore seems that in this test, in contrast to the social interaction test, the degree of anxiety is not necessarily reflected in changes in hippocampal tone. Because other brain regions control the escape components in the test, it is possible that changes in tone in these areas, rather than in the dorsal hippocampus, might reflect the extent of the phobic anxiety. The anxiolytic role of the endogenous cholinergic dorsal hippocampal system might play an important compensatory role, limiting the activation of the brain-aversive system mediating escape.

The close link between nicotinic modulation of anxiety and the $5\text{-}HT_{1A}$ receptor established for the dorsal hippocampus and the social interaction test may not hold for this brain region in other tests of anxiety. It will also be important to establish whether there is a similar close link between the nicotinic and $5\text{-}HT_{1A}$ modulation of anxiety in the social interaction test in other brain sites such as the lateral septum.

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