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# Neurobiological mechanisms by which nicotine mediates different types of anxiety

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**Abstract**

The effects of nicotine administration into the dorsal hippocampus and lateral septum provide further evidence that different neurochemical and neuroanatomical substrates control behaviour in different animal tests. Thus, in the social interaction test (a model of generalised anxiety disorder), bilateral administration of nicotine (1–4 μg) into both regions has anxiogenic effects in test conditions that generate moderate anxiety. The anxiogenic effects are mediated by a nicotine-evoked increase in 5-hydroxytryptamine (5-HT) release and are reversed by co-administration of the 5-$HT_{1A}$ receptor antagonist, *N*-(2-(6-(2-methoxyphenyl)-1-piperazinyl)ethyl)-*N*-(2-pyridyl)-cyclohexane carboxamide trichloride (WAY 100,635). On trial 1 in the elevated plus-maze (which models the escape components of panic disorder), nicotine is without effect when administered to the dorsal hippocampus, but has anxiogenic effects after lateral septal administration. On trial 2 in the elevated plus-maze (a model of specific phobia), nicotine (1 μg) has anxiolytic effects when administered to the dorsal hippocampus, but is ineffective (4 and 8 μg) in the lateral septum. 

*Keywords:* Nicotine; 5-$HT_{1A}$ receptor; Anxiety; Phobia; Septum, lateral; Hippocampus, dorsal

## 1. Introduction

The term anxiety disorder encompasses a number of conditions, including generalised anxiety disorder (GAD), panic disorder and specific (or simple) phobias. Different pharmacological treatments are effective in treating these three types of anxiety disorder. Thus, benzodiazepines are very effective in treating GAD, with 5-$HT_{1A}$ receptor agonists having a somewhat lower efficacy. In panic disorder, antidepressants are the most effective treatment, with benzodiazepines working only at high doses. In specific phobias, benzodiazepines are ineffective and, indeed, there is no really useful drug treatment for this disorder. This differing pattern of drug treatment suggests that these different anxiety disorders have different underlying neurobiological pathologies. It is not surprising, then, that factor analysis has provided evidence that measures obtained from different animal tests of anxiety measure quite independent factors, and thus the different animal tests are measuring different types, or states, of anxiety (File, 1992).

The hippocampus and septal nuclei are two regions of the limbic system that have long been implicated in the control of anxiety (Gray, 1982), but as will be seen in the following sections, these areas are not equally important in all animal tests of anxiety. Accumulating evidence from lesion studies and central drug administration has shown that different brain regions and neurotransmitters control behaviour in the different animal tests of anxiety (Menard and Treit, 1996; Treit and Menard, 1997, 1999). The effects of nicotine after bilateral administration to the dorsal hippocampus and lateral septum highlight the differing roles of the nicotinic cholinergic systems in modulating behaviour in three different animal tests of anxiety. The social interaction test is a model of GAD (File, 1980, 1997), trial 1 in the plus-maze measures the escape aspects of panic disorder (Graeff et al., 1993a,b) and trial 2 in the elevated plus-maze is a model of specific phobia (File and Zangrossi, 1993; File et al., 1993; Fernandes and File, 1996).

## 2. Effects in the social interaction test

In the social interaction test (File, 1980, 1997), the dependent variable is the time spent in social interaction (sniffing, following and grooming, boxing and wrestling)

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by pairs of rats, and the anxiety generated by this test can be manipulated by changing the light level and/or the rats' familiarity within the test arena. The greatest level of anxiety is generated by testing rats under high light, in an arena with which they are unfamiliar (HU); the lowest level of anxiety arises when rats are tested in a familiar arena, lit by low light (LF). Increases in social interaction are indicative of an anxiolytic effect and decreases indicate an anxiogenic response. In order to determine the specificity of these changes, locomotor activity is also measured to control for non-specific drug effects.

After systemic administration, low doses of nicotine (0.01–0.1 mg/kg) have anxiolytic effects, whereas higher (0.5 and 1 mg/kg) doses have anxiogenic effects (File et al., 1998b), when tested 30 min after i.p. injection, in test conditions that generate moderate levels of anxiety (HF: high light, familiar arena or LU: low light, unfamiliar arena). However, there is also a complex time-course of actions that can be seen following a single low dose of nicotine (0.1 mg/kg, s.c.). Five minutes after injection, there is an anxiogenic effect, that is followed by an anxiolytic effect at 30 min and a further anxiogenic effect at 1 h (Irvine et al., 1999). It is not known whether each of these effects is mediated by different brain regions, but there is evidence that distinct areas mediate the anxiogenic and anxiolytic effects. The anxiolytic effect of nicotine is mediated by the dorsal raphe nucleus (File et al., 1999a), whereas low doses of nicotine (5 and 10 ng) have anxiolytic actions. Low doses of nicotine are without effect in the dorsal hippocampus (Cheeta et al., 2000), but higher doses of nicotine have anxiogenic effects when injected into the dorsal hippocampus or the lateral septum (see below).

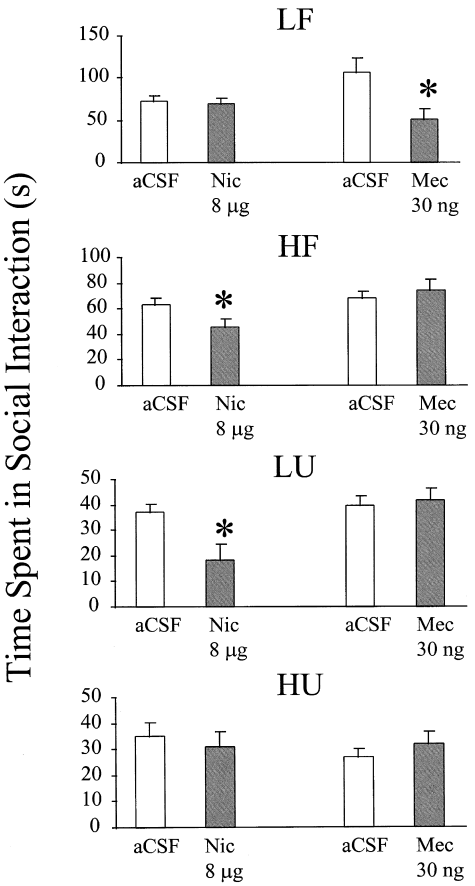


Fig. 1. Mean (±sem) time (s) spent in social interaction by rats injected bilaterally into the dorsal hippocampus with artificial CSF (aCSF), nicotine (Nic, 8μg) or mecamylamine (Mec, 30 ng) and tested in a low light, familiar arena (LF), a high light, familiar arena (HF), a low light, unfamiliar arena (LU) or a high light, unfamiliar arena (HU). $^{*}P < 0.05$ compared with control (aCSF) group. Reproduced with permission from File et al. (1998a,c).

### *2.1. Administration of nicotine and mecamylamine to the dorsal hippocampus*

The dorsal hippocampus plays an important role in controlling behaviour in the social interaction test and after bilateral administration, the benzodiazepine, midazolam, has an anxiolytic effect (Gonzalez et al., 1998), whereas the 5-$HT_{1A}$ receptor agonist, 8 hydroxy-2-(D-*n*-propylamino) tetracin (8-OH-DPAT), has an anxiogenic effect (Andrews et al., 1994; File et al., 1996).

Nicotine has an anxiogenic effect (indicated by a decrease in the time spent in social interaction, without any change in general locomotor activity) following direct bilateral administration into this brain region (File et al., 1998c). However, the effects are dependent on the test condition and anxiogenic effects are found only in the test conditions generating moderate anxiety (HF, 0.1–8 μg; LU, 8 μg; see Fig. 1). In contrast, the nicotinic channel blocker, mecamylamine (30–100 ng) has anxiogenic effects in the least anxiogenic test condition, LF (File et al., 1998a), but is ineffective in the other three conditions (File et al., 1998c; see Fig. 1).

It is, at first glance, surprising that both an agonist and an antagonist should have anxiogenic actions. In both cases, the anxiogenic action can be reversed with the 5-$HT_{1A}$ receptor antagonist, *N*-(2-(6-(2-methoxyphenyl)-1-piperazinyl)ethyl)-*N*-(2-pyridyl)-cyclohexane carboxamide trichloride (WAY 100,635) (File et al., 2000; Kenny et al., 2000a) and both mecamylamine and nicotine increase basal 5-HT release in the dorsal hippocampus (Kenny et al., 2000b). However, the paradox can be resolved if it is considered that the two drugs may be acting at different populations of nicotinic receptors (see Fig. 2). The evidence for this comes from the effects of glycine, which enhances the nicotine-stimulated 5-HT release, by a strychnine-sensitive mechanism, but blocks the mecamylamine-induced 5-HT release, by a strychnine-insensitive mechanism. The effects of mecamylamine are most readily seen in conditions of low anxiety, where there is high endogenous cholinergic tone and those of nicotine are expressed in conditions of increased serotonergic and glycinergic tone (see File et al., 2000).

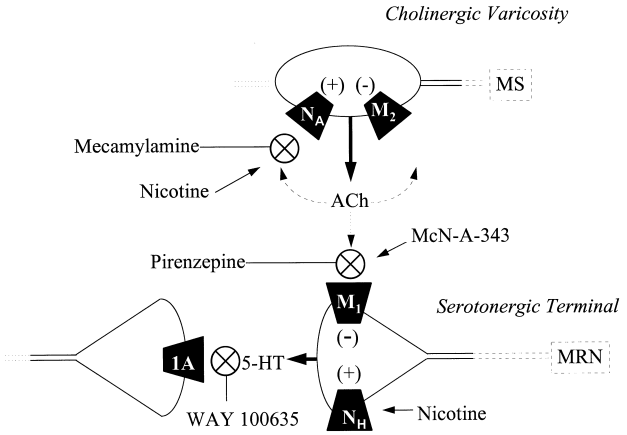


Fig. 2. Proposed model by which nicotinic and muscarinic receptor ligands modulate 5-HT release in the dorsal hippocampus. ACh — Acetylcholine; MS — medial septum; MRN — median raphe nucleus; $M_1$ — muscarinic $M_1$ receptor; $M_2$ — muscarinic $M_2$ receptor; 1A — post-synaptic $5\text{-HT}_{1A}$ receptor; $N_A$ — nicotinic autoreceptor; $N_H$ — nicotinic heteroreceptor; (+) indicates stimulatory action, (−) indicates inhibitory action on neurotransmitter release.

### *2.2. Effects of nicotine and mecamylamine in the lateral septum*

The lateral septum is another limbic area that modulates behaviour in the social interaction test and after bilateral administration, nicotine (4–8 μg) has an anxiogenic effect that can be reversed by co-administration of WAY 100,635 (Cheeta et al., 1999). The effects of mecamylamine injections into the lateral septum reveal a complex pattern, even within a single test condition (HF). At the lowest dose (15 ng), mecamylamine has an anxiolytic effect; at 30–50 ng, it is without significant effect, but is able to antagonise the anxiogenic effect of 4 μg nicotine (Ouagazzal et al., 1999a); and at 100 ng, it has an anxiogenic effect (see Fig. 3). This pattern of results suggests that the endogenous nicotinic cholinergic tone in the lateral septum has an anxiogenic modulatory action that can be antagonised by a low dose of mecamylamine and enhanced by nicotine. As the dose of mecamylamine is increased, there is action at a second population of nicotinic receptors and at 100 ng, this effect dominates. The endogenous cholinergic tone at these receptors serves an anxiolytic function. The cholinergic system interacts with many other neurotransmitter pathways and whilst there is evidence that nicotine's anxiogenic effects in the lateral septum are mediated via the 5-HT system, this has not been tested for mecamylamine. It is possible that the anxiogenic effect of mecamylamine is mediated by indirectly increasing 5-HT release (as shown in Fig. 2 for the dorsal hippocampus) and the anxiolytic effect is mediated by antagonising endogenous cholinergic tone on nicotinic heteroreceptors on the 5-HT terminal. However, interaction with other transmitters, such as noradrenaline, cannot be ruled out.

The two separate anxiogenic effects that are observed at different time-points in the social interaction test, at 5 min and 1 h, following sub-cutaneous administration of nicotine (Irvine et al., 1999) may be subserved by different brain regions, such as the dorsal hippocampus and lateral septum. Alternatively, there may be both acute and longer-term changes in the same regions that subserve anxiogenic effects at different time-points. This could be investigated by administering antagonists directly into these brain regions and determining their ability to block the anxiogenic effects of systemically administered nicotine at different time-points.

## 3. Effects of nicotine on trial 1 in the plus-maze

The elevated plus maze test is a widely used test of anxiety (Pellow et al., 1985), which consists of two open arms and two arms enclosed by 40-cm-high walls; the four

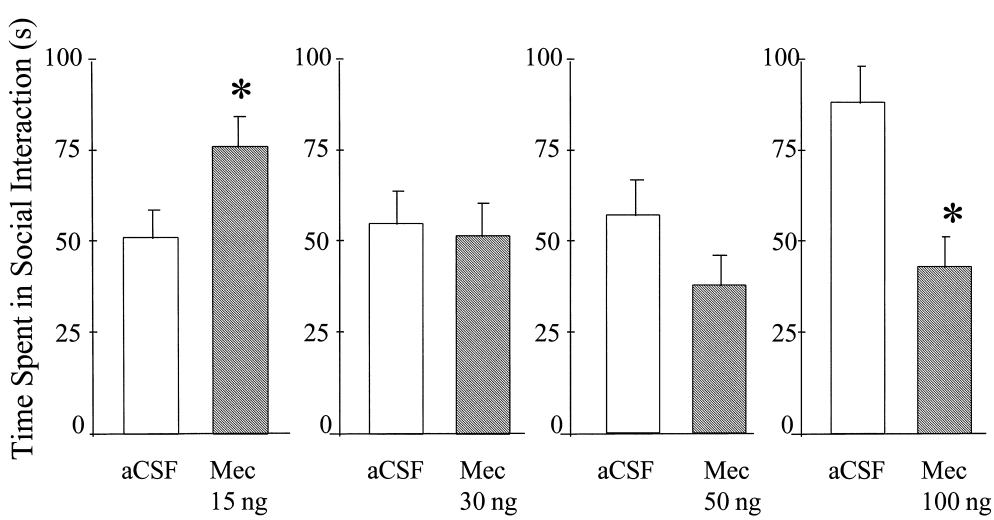


Fig. 3. Mean (±S.E.M.) time spent by rats tested in the high light, familiar (HF) test condition of the social interaction test after bilateral injection into the lateral septum of artificial CSF (aCSF) or mecamylamine (Mec, 15, 30, 50 or 100 ng). $^{*}P < 0.05$ compared with control (aCSF). Reproduced from Ouagazzal et al. (1999a).

arms intersect to form a central square. The entire maze is at an elevation of 50 cm above the floor. When naive rats are tested in the plus-maze (i.e., trial 1), it is the open aspects of the arms that is the factor generating anxiety (Treit et al., 1993). Anxiolytic effects are characterised by an increased percentage of entries onto, and an increased percentage of time spent on open arms; anxiogenic effects are characterised by decreases in these measures (Pellow and File, 1986; Pellow et al., 1985). Non-specific changes in locomotor activity are measured by changes in the number of closed arm entries (File, 1992).

After systemic administration in rats, both anxiolytic (Brioni et al., 1994) and anxiogenic (Ouagazzal et al., 1999a,b) effects of nicotine are reported on trial 1 in the plus-maze. The differences may lie in the strain of rats, the baseline scores or the previous history of the rats, factors that could affect the background cholinergic and serotonergic tone. Mecamylamine (0.1–0.5 mg/kg) has anxiolytic effects on trial 1 in the plus-maze, but higher doses (0.5 and 1 mg/kg) are ineffective (Kenny et al., 1998; Shytle et al., 1999; Table 1).

Evidence from central injections confirms that, as is the case in the social interaction test, it is again possible the anxiolytic and anxiogenic effects of nicotine in the plus-maze are mediated by different brain regions. Nicotine (4 μg) injected into the dorsal raphe nucleus has an anxiolytic effect, confirming that this brain region is mediating anxiolytic effects in at least two animal tests (File et al., 1999a). However, in contrast to the importance of the dorsal hippocampus in the social interaction test, this region plays little role in controlling behaviour on trial 1 in the plus-maze (see File et al., 2000). No effects of nicotine are found over a very wide dose-range (0.5 ng–8 μg) in this test after bilateral administration to the dorsal hippocampus (Ouagazzal et al., 1999b; Cheeta et al., 2000).

In contrast to the dorsal hippocampus, the lateral septum plays an important role in mediating behaviour in plus-maze naive rats. Lesions of the lateral septum have an anxiolytic effect (Menard and Treit, 1996) and Ouagazzal et al (1999a) found anxiogenic effects after direct injection of nicotine (1 and 4 μg) into the lateral septum. The anxiogenic effect of nicotine in the lateral septum is antagonised by co-administration of WAY 100,635 (Cheeta et al., 1999).

Table 1
Mean ± S.E.M. percentage time (s) spent on the open arms, percentage of open arm entries and number of closed arm entries made on trial 1 in the plus-maze by rats injected s.c. with vehicle or mecamylamine (Mec, 0.5 and 1 mg/kg)

| | Vehicle | Mec (0.5 mg/kg) | Mec (1 mg/kg) |
|---|---|---|---|
| Percentage time, open arms | 12.9 ± 4.1 | 32.3 ± 5.8[a] | 27.5 ± 9.4 |
| Percentage entries, open arms | 18.9 ± 3.9 | 35.3 ± 5.4[a] | 25.5 ± 5.6 |
| Closed arm entries | 9.3 ± 1.0 | 9.0 ± 0.7 | 10.6 ± 0.8 |

[a] $P < 0.05$ compared with vehicle (aCSF) control.

## 4. Effects of nicotine on trial 2 in the elevated plus-maze

A remarkable change occurs 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 (Treit et al., 1993), whereas by the second 5-min trial, it is the elevation of the arms that is the controlling factor (Fernandes and File, 1996). The type of anxiety measured on trials 1 and 2 is quite distinct (File, 1993; Rodgers and Johnson, 1995; Fernandes and File, 1996) and behaviour on trial 2 is insensitive to benzodiazepines (File, 1990; File et al., 1990; Rodgers and Shepherd, 1993; Rodgers et al., 1992). The lack of sensitivity to benzodiazepines is not due to habituation of anxiety since the scores on trial 2 either remain unchanged (Pellow et al., 1985; File 1990; Taukulis and McKay, 1992) or decrease, indicating enhanced anxiety (Rodgers and Shepherd, 1993; File and Gonzalez, 1996; Gonzalez and File, 1997); nor is there any habituation from trial 1 to trial 2 of the corticosterone stress response (File et al., 1994; Holmes and Rodgers, 1998). 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 (File and Zangrossi, 1993; File et al., 1998b, 1999b). Fear of heights is the most common of specific phobias (Goisman et al., 1998) and benzodiazepines are ineffective against specific phobias (Marks, 1987; Tyrer, 1989).

After systemic administration, nicotine (0.1 mg/kg) causes a non-significant increase in the percentage of time in the open arms, and higher doses (0.5 and 1 mg/kg) have an anxiogenic effect (Ouagazzal et al., 1999b). However, after bilateral injection into the lateral septum, nicotine is without effect at doses of 4–8 μg (File et al., 1999a). This highlights an important difference between the brain regions controlling behaviour on trials 1 and 2 in the plus-maze. This is further exemplified by the finding that nicotine (1 μg) has an anxiolytic effect after dorsal hippocampal administration on trial 2, yet is ineffective on trial 1 (Ouagazzal et al., 1999b). The nicotinic receptor antagonist, mecamylamine, and the muscarinic $M_1$ receptor antagonist, pirenzepine, have anxiogenic effects on trial 2 after dorsal hippocampal administration (File et al., 1998a), whereas the muscarinic $M_1$ receptor agonist, McN-A-343 has an anxiolytic effect (File et al., 2000). Thus, although the dorsal hippocampus plays a role in both the social interaction test and trial 2 in the elevated plus-maze, the direction of nicotine's effects is opposite in the two tests. The 5-$HT_{1A}$ receptor agonist, 8-OH-DPAT, has an anxiogenic effect in both these tests when injected into the dorsal hippocampus (Andrews et al., 1994; File et al., 1996), which suggests that stimulation of 5-$HT_{1A}$ receptors is anxiogenic in both tests. The anxiogenic effect of nico-

tine in the social interaction test seems to be mediated via stimulation of 5-$HT_{1A}$ receptors, but the mechanism underlying nicotine's anxiolytic action on trial 2 remains unknown.

Because it is possible to see clear anxiogenic effects of dorsal hippocampal administration of mecamylamine and pirenzepine on trial 2, this suggests that there is a relatively high cholinergic tone. The endogenous serotonergic tone (or at least the state of 5-$HT_{1A}$ receptor activation) would appear to be relatively low, since it is possible to see the effects of further activation by 8-OH-DPAT. Thus, on trial 2, there is high cholinergic tone and low 5-$HT_{1A}$ receptor activation, conditions that in the social interaction test are characteristic of the lowest level of anxiety, that is, that generated by the LF test condition. It certainly does not seem to be the case that trial 2 in the plus-maze generates a low level of anxiety and, in general, the scores indicate a higher anxiety level on trial 1 than trial 2 (e.g., Rodgers and Shepherd, 1993; Gonzalez and File, 1997). Furthermore, the corticosterone concentration is as high on trial 2 as on trial 1 (File et al., 1994; Holmes and Rodgers, 1998). This suggests that, whereas in the social interaction test, the endogenous dorsal hippocampal tone reflects the degree of anxiety, on trial 2 in the elevated plus-maze, this may not be the case. It is known that several other brain areas are important in controlling behaviour on trial 2. For example, the basolateral nucleus of the amygdala is crucial to the acquisition of the fear of heights (File et al., 1998b) and the dorsomedial hypothalamus is crucial to the expression of the escape components of the response on trial 2 (File et al., 1999b). Phobic behaviour has two distinct components, avoidance of the phobic object and, if confronted with it, intense anxiety and escape from it (Marks, 1987). It is possible that the dorsal hippocampus is more concerned with the avoidance component of the response on trial 2 and the dorsal hypothalamus with the escape component. The nicotinic cholinergic system in the dorsal hippocampus may play an important compensatory anxiolytic role, limiting the activation of other pathways both within and outside the hippocampus.

## 5. Conclusions

The effect of mecamylamine in the various tests reveals that the endogenous cholinergic tone differentially modulates anxiety in these tests and that the cholinergic tone may play different roles in different brain structures. In the social interaction test, the endogenous tone in the dorsal hippocampus plays an anxiolytic role in the LF test condition, but seems to reduce in importance as the test conditions become more anxiogenic. In the lateral septum, it is possible to detect a functional endogenous tone in the HF test condition. At some nicotinic cholinergic receptors in the lateral septum, the endogenous tone serves an anxiolytic function (as shown by the anxiogenic effect of 100 ng mecamylamine), whereas at another population of receptors, it plays an anxiogenic role (as indicated by the anxiolytic effects of 15 ng mecamylamine). It would be important to determine whether one or other of these actions dominates in test conditions that generate higher or lower levels of anxiety.

Table 2
Effects on anxiety of dorsal hippocampal and lateral septal injections of nicotine
↑, Increased anxiety; =, no behavioural effect; ↓, decreased anxiety.

| Anxiety test | Dorsal hippocampus | Lateral septum |
|---|---|---|
| Social interaction | ↑ | ↑ |
| Trial 1 | = | ↑ |
| Trial 2 | ↓ | = |

When rats are placed in the plus-maze on trial 1, there is no evidence of a functional cholinergic tone in the dorsal hippocampus and both mecamylamine and pirenzepine are without effect. However, the effects of systemic administration of mecamylamine suggest that an anxiogenic cholinergic tone predominates in this test. In contrast, at least in the dorsal hippocampus, the endogenous cholinergic tone plays an anxiolytic role on trial 2 in the elevated plus-maze, as reflected by the anxiogenic effects of both mecamylamine and pirenzepine.

As can be seen from Table 2, the pattern of nicotine's effects on anxiety after administration to the dorsal hippocampus differs in all three of the tests that we have explored. Nicotine has anxiogenic effects in the social interaction test, is inactive on trial 1 in the plus-maze and has an anxiolytic effect on trial 2 in the elevated plus-maze. This provides further evidence for an important difference in the neurochemical mediation of behaviour on trials 1 and 2 in the plus-maze. This point is nicely illustrated by comparing the effects of nicotine in the dorsal hippocampus and lateral septum. In this case, there is a double-dissociation of effects, with nicotine inactive in the dorsal hippocampus, but active in the lateral septum on trial one, but active in the dorsal hippocampus and inactive in the lateral septum on trial 2.

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