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Research report

# Temporary inhibition of dorsal or ventral hippocampus by muscimol: Distinct effects on measures of innate anxiety on the elevated plus maze, but similar disruption of contextual fear conditioning

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## HIGHLIGHTS

- On elevated plus maze, ventral hippocampal muscimol reduced locomotion.
- Reduced locomotion was possibly accompanied by anxiolytic effects.
- In contrast, dorsal hippocampal muscimol effects were consistent with anxiogenesis.
- Ventral and dorsal hippocampal muscimol impaired contextual fear conditioning.
- Neither ventral nor dorsal muscimol affected tone fear conditioning.

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## ABSTRACT

Studies in rats, involving hippocampal lesions and hippocampal drug infusions, have implicated the hippocampus in the modulation of anxiety-related behaviors and conditioned fear. The ventral hippocampus is considered to be more important for anxiety- and fear-related behaviors than the dorsal hippocampus. In the present study, we compared the role of dorsal and ventral hippocampus in innate anxiety and classical fear conditioning in Wistar rats, examining the effects of temporary pharmacological inhibition by the GABA-A agonist muscimol (0.5 ug/0.5 ul/side) in the elevated plus maze and on fear conditioning to a tone and the conditioning context. In the elevated plus maze, dorsal and ventral hippocampal muscimol caused distinct behavioral changes. The effects of ventral hippocampal muscimol were consistent with suppression of locomotion, possibly accompanied by anxiolytic effects, whereas the pattern of changes caused by dorsal hippocampal muscimol was consistent with anxiogenic effects. In contrast, dorsal and ventral hippocampal muscimol caused similar effects in the fear conditioning experiments, disrupting contextual, but not tone, fear conditioning.



## 1. Introduction

Studies examining the effects of lesion or pharmacological manipulations of the hippocampus in rats have provided compelling evidence that the hippocampus is important for unconditioned anxiety/fear responses, as well as the formation and expression of conditioned fear responses to elemental (e.g., auditory) and contextual stimuli[1] Moreover, the weight of evidence from studies using separate ventral or dorsal hippocampal manipulations suggests that the ventral hippocampus plays a rather general role in unconditioned anxiety and conditioned fear, whereas dorsal hippocampal contributions are more restricted to specific mnemonic aspects of fear conditioning, such as context learning; this is consistent with the ventral hippocampus featuring stronger direct connectivity to amygdala and hypothalamus, key components of the brain's anxiety and fear circuit, whereas the dorsal hippocampus is more closely linked to parts of the entorhinal cortex that are implicated in visuo-spatial information encoding [1–12].

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[1] Anxiety and fear both refer to responses to aversive stimuli and situations. Anxiety is more commonly used to refer to unconditioned, rather tonic, responses to more diffuse stimuli or situations associated with behavioral conflict and uncertainty, whereas fear commonly refers to rather phasic responses to stimuli associated with explicit danger (compare [47], and [6], and references therein).

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The present paper reports three experiments, in which we compared further the contributions of dorsal and ventral hippocampus to unconditioned anxiety and conditioned fear. We examined the effects of bilateral functional inhibition of neurons within dorsal or ventral hippocampus by local microinfusion of the GABA-A agonist muscimol (0.5 μg/0.5 μl/side) on measures of unconditioned anxiety on the elevated plus maze (Experiment 1) and on the formation of conditioned fear (measured as freezing) to a tone or the conditioning context (Experiments 2 and 3). The elevated plus maze experiment (Experiment 1) addressed the hypothesis that ventral hippocampal muscimol would cause more pronounced anxiolytic effects than dorsal muscimol. This hypothesis is consistent with the idea that the ventral hippocampus plays a more important role in unconditioned anxiety than the dorsal hippocampus, which is based on wide range of evidence [6,8,10]). More specifically, ventral cytotoxic lesions have been found to cause more pronounced anxiolytic effects than dorsal lesions on a variety of measures of innate anxiety, including elevated plus maze measures [4,6,13], and ventral infusion of the local anesthetic lidocaine (a sodium channel blocker inactivating neurons and fibers of passage) significantly increased the proportion of open-arm entries on the elevated plus maze test, whereas dorsal lidocaine had no significant effect [14]. Moreover, ventral, but not dorsal, hippocampal muscimol reduced unconditioned fear, as assessed by the shock-probe burying test [15]. However, even though one study reported that dorsal hippocampal muscimol reduced measures of unconditioned anxiety on the elevated plus maze [16], the effects of dorsal and ventral hippocampal muscimol infusions on the elevated plus maze remain to be compared directly. Furthermore, in the present study, the effects of dorsal and ventral hippocampal muscimol infusions are examined alongside the effects of these manipulations on fear conditioning (Experiments 2 and 3), allowing a direct comparison. In the fear conditioning experiments (Experiment 2 and 3), we aimed to corroborate our previous finding that ventral hippocampal muscimol (1 μg/0.5 μl/side) disrupts contextual, but not tone, fear conditioning [17] and to extend this finding by demonstrating similar effects of dorsal hippocampal muscimol. Such an outcome would be consistent with the idea that contextual fear conditioning requires dorsal hippocampal mechanisms mediating the formation of context representations, and ventral hippocampal mechanisms relating the context representations to fear processing via subcortical structures, including the amygdala [2,5,6,11,17,18]. While the ventral hippocampus has also been implicated in tone fear conditioning [3,6], ventral hippocampal muscimol did not significantly reduce tone fear conditioning in our previous study (even though there was a numerical reduction), and we argued that partial inhibition of neuronal activity in the ventral hippocampus via GABA-A receptor stimulation may not sufficiently interfere with ventral hippocampal processing to affect tone fear conditioning (in contrast, more general ventral hippocampal inactivation by the sodium channel blocker tetrodotoxin markedly impaired tone fear conditioning) [17]. Following our initial 2001 study [17], a number of studies examined the effects of hippocampal muscimol infusions on fear conditioning, with somewhat discrepant outcomes. Maren and Holt [7] reported that ventral hippocampal muscimol (0.25 μg/0.25 μl/side) disrupted tone, but not contextual (background), fear conditioning, whereas dorsal infusions had no effect. Consistent with two main findings by Maren and Holt [7], additional studies reported that dorsal hippocampal muscimol (0.5 μg/0.5 μl/side; [19]) and muscimol infusion into the ventral hippocampus (subiculum; 0.5 μg/1 μl/side; [20]) did not cause anterograde contextual fear conditioning deficits. Such absence of anterograde contextual fear conditioning deficits following hippocampal muscimol (and also lesions) was explained by the competition hypothesis [20–22]. This hypothesis suggests that, while hippocampal mechanisms are normally important for contextual fear conditioning, they compete with an extra-hippocampal system that can also support contextual fear conditioning, albeit less efficiently; the hippocampus normally suppresses the alternative system, but this suppression is released during hippocampal inactivation or inhibition, so that the extra-hippocampal system can support contextual fear conditioning. Most recently, however, Esclassan et al. [23] reported that ventral hippocampal muscimol (0.25 μg/0.25 μl/side) disrupted both tone and contextual fear conditioning, whereas dorsal muscimol selectively reduced contextual fear conditioning, and Wang et al. [24] also reported that dorsal hippocampal muscimol (0.5 μg/0.5 μl/side) impaired contextual fear conditioning [24]. Considering the different findings made in different laboratories, we found it important to re-examine the anterograde effects of ventral hippocampal muscimol infusions [17] and to compare directly the effects of ventral and dorsal hippocampal muscimol on fear conditioning in our laboratory.

## 2. Materials and methods

### 2.1. Subjects

A total of 40 adult male Wistar rats (Zur:WIST[HanIbm], Research Unit Schwerzenbach, Schwerzenbach, Switzerland), weighing about 250 g and aged about 2–3 months at the time of surgery, were used in this study. They were housed in groups of four per cage under a reversed light–dark cycle (lights on: 19:00–07:00) in a temperature (21 ± 1 °C) and humidity (55 ± 5%) controlled room. All animals were allowed free access to food and water. Eighteen rats received bilateral implantation of guide cannulae aiming at the dorsal hippocampus and twenty-two rats received bilateral implantation of guide cannulae aiming at the ventral hippocampus. After surgery, all rats were caged singly. Starting one day before surgery and throughout the study, all rats were handled daily. Behavioral testing was carried out in the dark phase of the cycle, between 9 and 18 h. Principles of laboratory animal care (NIH publication no. 86-23, revised 1985) and Swiss regulations for animal experimentation were followed.

### 2.2. Apparatus and procedures

#### 2.2.1. Surgery

Rats were anesthetized (i.p.) with Nembutal (sodium pentobarbital, Abbott Labs, North Chicago, IL; 50 mg/kg body weight), together with a mixture of midazolam hydrochloride (Dormicum®, Hoffman–LaRoche, Switzerland; 2 mg/kg body weight) and medetomidin hydrochloride (Dormitor, Orion Corporation, Espoo, Finland; 0.15 mg/kg body weight) given intramuscularly. Then their head was placed in a Kopf stereotaxic frame. After application of a local anesthetic (lidocaine), the scalp was incised to expose the skull. Bregma and lambda were aligned in the same horizontal plane. A small hole (1.5 mm in diameter) was drilled on each side of the skull to reveal the dura covering the cortex overlying the hippocampus. Stainless steel guide cannulae (26 G, 9 mm or 7 mm for ventral or dorsal hippocampus, respectively) in a custom-made Perspex holder were implanted bilaterally into the brain aiming above the ventral (−5.2 mm posterior and ±5.0 mm lateral to bregma, and −5.0 mm ventral to dura) or dorsal (−3.0 mm posterior and ±1.5 mm lateral to bregma, and −2.5 mm ventral to dura) hippocampus, using the same coordinates as in previous studies [5,17,25,26]. The guide cannulae were fixed to the skull with three anchoring skull screws and dental cement. Stainless steel stylets (34 G) extending 0.5 mm beyond the tips of the guide cannulae were placed inside the guide cannulae to prevent occlusion. After surgery, rats were allowed to recover for five days during which the