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Research report

# Ventral tegmental area cholinergic mechanisms mediate behavioral responses in the forced swim test

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## HIGHLIGHTS

- Systemic or VTA physostigmine increases immobility in the forced swim test (FST).
- VTA mecamylamine or scopolamine decreases immobility time in the FST.
- VTA scopolamine blocks the effects of VTA physostigmine in the FST.
- VTA mecamylamine does not alter the effect of VTA physostigmine in the FST.

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## ABSTRACT

Recent studies revealed a causal link between ventral tegmental area (VTA) phasic dopamine (DA) activity and pro-depressive and antidepressant-like behavioral responses in rodent models of depression. Cholinergic activity in the VTA has been demonstrated to regulate phasic DA activity, but the role of VTA cholinergic mechanisms in depression-related behavior is unclear. The goal of this study was to determine whether pharmacological manipulation of VTA cholinergic activity altered behavioral responding in the forced swim test (FST) in rats. Here, male Sprague-Dawley rats received systemic or VTA-specific administration of the acetylcholinesterase inhibitor, physostigmine (systemic; 0.06 or 0.125 mg/kg, intra-cranial; 1 or 2 μg/side), the muscarinic acetylcholine receptor (AChR) antagonist scopolamine (2.4 or 24 μg/side), or the nicotinic AChR antagonist mecamylamine (3 or 30 μg/side), prior to the FST test session. In control experiments, locomotor activity was also examined following systemic and intra-cranial administration of cholinergic drugs. Physostigmine administration, either systemically or directly into the VTA, significantly increased immobility time in FST, whereas physostigmine infusion into a dorsal control site did not alter immobility time. In contrast, VTA infusion of either scopolamine or mecamylamine decreased immobility time, consistent with an antidepressant-like effect. Finally, the VTA physostigmine-induced increase in immobility was blocked by co-administration with scopolamine, but unaltered by co-administration with mecamylamine. These data show that enhancing VTA cholinergic tone and blocking VTA AChRs has opposing effects in FST. Together, the findings provide evidence for a role of VTA cholinergic mechanisms in behavioral responses in FST.



## 1. Introduction

Depression affects approximately 1 in 6 individuals in the United States [1] and there is a critical need for improved understanding of the neurocircuitry of depression and a need for more effective therapeutic interventions. Individuals with unipolar and bipolar depression commonly show impairments in goal-directed and motivated behaviors, exhibited as psychomotor slowing, anergia, and fatigue [2–4], that are highly resistant to treatment [5–7]. A large body of research in humans, non-human primates and rodents has shown that the mesolimbic dopamine (DA) system, including the ventral tegmental area (VTA) and the nucleus accumbens (NAc), plays a critical role in motivated behaviors in humans [8–10]. Further, mesolimbic dopamine deficits are associated with major depressive disorder (MDD) in humans and have also been observed in preclinical genetic and behavioral models of depression [11–13]. Recent findings in rodent models have revealed a

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causal link between phasic DA activity in the VTA to NAc pathway in specific pro-depressive and antidepressant-like behavioral phenotypes [14,15]. Such findings raise the possibility that processes which regulate mesolimbic DA activity may mediate depression-related behavior. Indeed, previous work has demonstrated that midbrain cholinergic activity powerfully regulates DA activity [16–19] and DA-dependent drug-seeking behaviors [20–22]. However, the role of VTA cholinergic mechanisms in depression and depression-related behavior is poorly understood.

The cholinergic hypothesis of depression is strongly supported by extensive experimental evidence from both humans [23–27] and rodents [28–32]. Specifically, manipulations that increase brain cholinergic tone lead to pro-depressive effects [23–25,29,32,33], while administration of either nicotinic or muscarinic acetylcholine receptor (AChR) antagonists leads to antidepressant-like effects [26,28,34–38]. Recent investigations of cholinergic mechanisms in depression have focused on key brain structures implicated in depression and other mood disorders, including the prefrontal cortex (PFC) [38–40], the hippocampus [28,29,41], and the NAc [42–44]. However, the field lacks understanding of the role of VTA cholinergic activity in depression, despite the fact that VTA cholinergic receptor mechanisms powerfully regulate phasic DA activity that is causally linked to depression-related behavioral responses to stress. Given that the VTA sends projections and receives input from the PFC, hippocampus, and NAc, examining VTA cholinergic mechanisms in depression-related behavior will also provide better understanding of the neurocircuitry of depression and the role of acetylcholine within this circuit.

Here, we sought to determine whether VTA acetylcholine and AChR activity in male Sprague-Dawley rats mediated behavioral responses in the forced swim test (FST). The goal of our study was to identify the role of midbrain mechanisms in behavioral responses in FST – in order to provide insight of the underlying neurobiological processes that mediate behavioral responses to stress. Using behavioral pharmacology, we found that intra-VTA infusion of nicotinic or muscarinic AChR antagonists decreased immobility time in FST – consistent with an antidepressant-like effect, while VTA infusion of the acetylcholinesterase inhibitor, physostigmine, led to an increase in immobility time that was dependent upon muscarinic AChR activation. The results of study reveal that VTA cholinergic manipulations robustly modulate behavioral responses in FST, independent of potential non-specific locomotor effects, and suggest that future investigation in this area can provide new understanding of the role of VTA cholinergic mechanisms in depression-related behaviors.

## 2. Materials and methods

### 2.1. Animals and surgery

Across all experiments, a total of 137 male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA, USA) were used. Rats were housed in pairs in a colony maintained at 22–24 °C with a 12 h light/12 dark cycle (lights on at 07:00) and were allowed 1 week to acclimate to the facility prior to any surgical procedures. Food and water were available ad libitum in the home cages at all times. Prior to surgery, rats were anesthetized with ketamine HCl (100 mg/kg, i.p., Sigma Aldrich, USA) and xylazine (10 mg/kg, i.p., Sigma Aldrich, USA) and placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA) for implantation of intra-cranial cannula. All coordinates were obtained from the rat brain atlas [45] with anteroposterior (AP), mediolateral (ML) and dorsoventral (DV) positions referenced from Bregma. A bilateral cannula spaced 1 mm apart (Plastics One, Roanoke, VA, USA) was placed 1 mm above the VTA (AP −5.2 mm, ML ±0.5 mm, DV −7.0 mm from dura) and secured using screws (Gexpro, High Point, NC) and dental cement (Dentsply, Milford, DE, USA). For the dorsal control site experiment, bilateral cannula were placed 3 mm above the VTA (AP −5.2 mm, ML ±0.5 mm, DV −5.0 mm from the dura). After surgery, rats were singly housed and allowed to recover for 5–7 days before testing began. Animal protocols were approved by Yale University Institutional Animal Care and Use Committee (IACUC) and performed in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals.

### 2.2. Drug administration

In preparation for brain-region specific drug delivery, bilateral internal cannula containing the drug were inserted into the guide cannula and extended 1 mm beyond the guide cannula to target the VTA (−8.0 mm from dura) or the dorsal control site (−6.0 mm from dura). Drugs were delivered in a 0.5 μL total volume over 1 min via a micro-infusion pump and syringe (25 gauge, Hamilton Syringe, Reno, NE, USA). After the 1 min drug infusion, the internal cannulae were left in place for an additional 1 min to allow for complete diffusion of drug into the brain tissue. The muscarinic AChR antagonist, scopolamine (Sigma Aldrich, St. Louis, MO), the nicotinic AChR antagonists, mecamylamine (Sigma Aldrich, St. Louis, MO), and the acetylcholinesterase inhibitor, physostigmine (VWR, Bridgeport, NJ) were each dissolved in 0.9% saline and infused at doses that we and others have previously shown to modulate behavior and phasic DA release when infused into the VTA [19,20,22,46]. Physostigmine, which inhibits acetylcholinesterase and prevents acetylcholine degradation, was used to enhance acetylcholine levels. Behaviorally relevant doses of systemic physostigmine were based on previously published work demonstrating pro-depressive effects of systemic administration and on pilot experiments that identified doses of physostigmine that did not alter baseline locomotor activity [29,47,48].

### 2.3. Forced swim test

The forced swim test (FST) was performed similar to protocols previously described by others [38,43,49–51]. During the pre-test, no pharmacological manipulation was given and behavior was not recorded. In the pre-test, rats were individually placed into a clear polypropylene, cylindrical water tank (diameter 30 cm; height 60 cm; water depth > 40 cm; water temperature between 23 and 26 °C) for 15 min, to establish a stable baseline of immobility for the subsequent test. The FST test session occurred during the second swim session, which took place 24 h after the pre-test. For systemic drug administration, physostigmine or vehicle was administered by intraperitoneal (i.p.) injection 20 min prior to the 10 min FST (Fig. 1a). For VTA-specific infusion, rats were placed into the water tank immediately after drug infusion and immobility was scored during the last 6 min of the 10 min test session (Fig. 2a). Thus, there was a 4 min wait time between VTA drug infusion and the analysis of immobility time. This wait time is consistent with the time course for physiological responses to VTA drug infusion, as we have previously demonstrated that VTA infusion of cholinergic drugs alters dopamine release within 3 min of infusion – with effects that last up to 2 h [19,20]. Each rat was randomly assigned to a specific drug administration group. FST was recorded by video camera and immobility was defined as an interruption of swimming behavior, when rats showed a lack of hind and fore paw paddling. Thus, scoring of immobility time started when rats assumed a passive floating position, using only minimal movements required to keep their heads above water. For FST analysis, each test session was quantified by stopwatch by an experimenter blind to the treatment condition. Tank water was cleaned after each rat. At the end of the test session, rats were dried with a towel and placed in a warmed