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Chun Hu April 8, 2015

Effect of M_1 Muscarinic Receptor Activators on Locomotion in Rats

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An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
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ABSTRACT

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By Chun Hu

The hippocampus is a brain region crucial for learning and memory and is impacted in Alzheimer's disease (AD). Drugs that target specific muscarinic acetylcholine receptors represent potential therapies for improving memory in AD. Throughout the brain, M_1 is the predominant post-synaptic mAChR that mediates excitatory metabotropic effects of the neurotransmitter acetylcholine. A recent study in rats found that systemic administration of the M_1-specific allosteric agonist VU0364572 enhanced spatial encoding ability as measured by spatial representations of hippocampal place cells (Lebois, 2014). However, place cell activity is known to correlate with self-motion cues such as running speeds. Thus, VU0364572 may have impacted place cells directly via activation of M_1 receptors in the hippocampus (or connected regions) or indirectly by influencing locomotion. The present study reanalyzed the locomotor activity from the previous study (Lebois, 2014). We found that the M_1 allosteric agonist, VU0364572, the M_1 potentiator, BQCA and the FDA-approved acetylcholinesterase inhibitor, Donepezil all had no significant impact on locomotor parameters including running speeds, percent time spent stationary and thigmotaxic behavior. Our findings suggest that influence of VU0364572 on neural activity in hippocampal pyramidal neurons could not simply be accounted for by differences in locomotion.

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Finally, I owe the deepest gratitude to my parents, Zhongyi and Yulan. You have put unyielding faith in me along every step of my undergraduate career. I am forever grateful for the sacrifices you have made throughout your lives to provide the best possible upbringing for me.

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INTRODUCTION

The hippocampus is crucial for learning and memory and is among the first brain regions to become dysfunctional in conditions such as Alzheimer's disease (AD) (Holtzman, Morris, & Goate, 2011). A large body of literature has shown that the cholinergic system is affected early in the disease progression of AD (Coyle, Price, & DeLong, 1983; Davies & Maloney, 1976; Drever, Riedel, & Platt, 2011). Specifically, the nuclei of the cholinergic basal forebrain, which includes the nucleus basalis of Meynert, the medial septum, diagonal band of Broca and the lateral septum, experienced heavy neuronal loss (~80%) in advanced AD (Mufson, Bothwell, & Kordower, 1989; Whitehouse et al., 1982). It is known that the medial septum/diagonal band of Broca provides key cholinergic input to the hippocampus (Amaral & Kurz, 1985; Milner, Loy, & Amaral, 1983). Therefore, it is reasonable to conclude that the medial septum plays a key role in shaping memory processing mediated by the hippocampus. The observations of the dramatic loss of cholinergic neurons in AD patients and the decrease in enzymatic activity for acetylcholine (ACh) synthesis have prompted attempts to treat AD by pro-cholinergic treatments, including acetylcholinesterase inhibitors (AChEIs) such as donepezil, physostigmine and rivastigmine (Colovic, Krstic, Lazarevic-Pasti, Bondzic, & Vasic, 2013). AChEIs are also one the two classes of currently FDA-approved AD therapeutics, with N-methyl-D-aspartate receptor (NMDAR) antagonists being the other class. AChEIs act by inhibiting acetylcholinesterase, the enzyme responsible for degrading ACh, thereby maintaining ACh concentration within the synapse. However, the mechanism of action of AChEIs is non-selective by definition, which is in part

responsible for a variety of adverse side effects including gastrointestinal side effects, extrapyramidal movement disorders, urination, defecation, salivation, lacrimation, cardiorespiratory effects, and sleep disturbances (Thompson, Lanctot, & Herrmann, 2004). The peripheral side effects due to ACh off-target effects from the non-selective pharmacological profile of AChEIs called for efforts to develop selective compounds for acetylcholine receptors.

Muscarinic acetylcholine receptors (mAChRs) are an essential component of the cholinergic system in that mAChRs mediate the metabotropic effects of acetylcholine (Halliwell, 1990). Five mAChR subtypes have been cloned ($M_{1\text{-}5}$) (Caulfield, 1993). M_1 mAChRs couple via G_q G-proteins to increase intracellular calcium and mediate excitatory neuromodulatory effects of ACh (Langmead, Watson, & Reavill, 2008). M_1 activates phospholipase C (PLC) in order to cause release of calcium from endoplasmic reticulum via protein kinase C (PKC) (Wess, 1996a). M_1 is the predominant postsynaptic mAChR subtype expressed in the brain and periphery (Shapiro, Wakimoto, Subers, & Nathanson, 1989; Wess, 1996b) and is heavily enriched in the hippocampus, the amygdala, the neocortex and the striatum (Levey, 1996). A large body of literature has demonstrated that M_1 activation could induce long-term potentiation (LTP) of hippocampal excitatory synaptic responses (Abe, Nakata, Mizutani, & Saito, 1994; Auerbach & Segal, 1996; Blitzer, Gil, & Landau, 1990; Burgard & Sarvey, 1990; Markram & Segal, 1990; Shinoe, Matsui, Taketo, & Manabe, 2005). A study with M_1 knockout mice has also indicated key roles of M_1 for memory consolidation and working memory (Anagnostaras et al., 2003). In the same study (Anagnostaras et al., 2003), M_1 knockout mice were also selectively impaired in hippocampal LTP induction when

compared to wild-type animals. Furthermore, M_1 signaling was found to potentiate NMDA currents in hippocampal pyramidal cells, which is important for memory consolidation (Lebois et al., 2009; Marino, Rouse, Levey, Potter, & Conn, 1998). Thus, M_1-specific drugs represent a promising therapeutic target for neurodegenerative diseases such as AD (Langmead et al., 2008).

Besides non-selective pan-mAChR activator AChEIs, two other classes of drugs activate M_1: M_1 agonists and M_1 positive allosteric modulators (PAM). While AChEIs represent the current standard of care for AD (Ibach & Haen, 2004), their efficacy is limited because they activate all mAChR subtypes, some of which functionally oppose one another (Langmead et al., 2008). Bodick and colleagues (1997) showed that mAChR activators, such as the dual M_1/M_4 agonist Xanomeline, improved memory in the clinic, but displayed relatively low M_1 receptor selectivity, resulting in unacceptable peripheral gastrointestinal side effects, believed to be M_3 mediated (Bodick et al., 1997). BQCA represented a breakthrough due to its mechanism of action as a type of PAM for mAChRs. BQCA, as a PAM, is a compound that increases the action of the orthosteric agonist (in this case ACh) by binding at an allosteric site and causing a change in receptor conformation. BQCA was found to be active in a reversal learning paradigm in a transgenic mouse model of AD, demonstrating the *in vivo* utility of this compound for selectively activating M_1 (Shirey et al., 2009). However, the efficacy of BQCA might be impaired in diseases such as AD where endogenous ligand (ACh) level is low because the action of BQCA depends on level of orthosteric agonist (ACh). Furthermore, central penetrance and low solubility still remained a problem for the development of M_1 PAMs.

Recently, a highly potent and selective allosteric agonist for M_1, VU0364572, has been developed (Lebois et al., 2011). VU0364572 was tested to be highly water soluble and orally bioavailable. Moreover, VU0364572 demonstrated a clean ancillary pharmacological profile in that it showed no significant off-target interactions with 68 different ion channels and G-protein coupled receptors (Lebois et al., 2011). In contrast to orthosteric activators (endogenous ligands, such as ACh), allosteric activators bind at a site that is topographically-distinct from orthosteric site to activate M_1 (Lebois et al., 2010). VU0364572 potentiated NMDA currents in hippocampal pyramidal cells to promote synaptic plasticity (Lebois et al., 2011). VU0364572 has been found to enhance memory in the Morris water maze (a test of hippocampal spatial memory) at doses of 0.1 and 1.0 mg/kg. VU0364572 was also demonstrated to potentiate LTP at lower concentrations while inducing LTD at higher concentrations at CA3-CA1 synapses (Digby et al., 2012).

More recent work (Lebois, 2014) found that VU0364572 dose-dependently improved the ability of the hippocampus to encode novel spatial information via influencing hippocampal place cells (Figure 1). Hippocampal place cells are pyramidal neurons that fire in response to specific locations to provide a population representation of location and self-motion (Muller & Kubie, 1987; Wilson & McNaughton, 1993). It has been proposed that self-motion cues play an important role in modulating place field formation (McNaughton et al., 1996; Samsonovich & McNaughton, 1997). In particular, place cell activity is known to correlate with self-motion cues such as running speed (Terrazas et al., 2005) and head direction (McNaughton, Chen, & Markus, 1991) of animals. Therefore, an important remaining question for how to interpret the

aforementioned neural findings (Lebois, 2014) is the extent to which VU0364572 impacted locomotor activity in rats. In particular, it is possible that VU0364572 only influenced place field activity indirectly via a more general influence on locomotion.

Very little is known about the effect of selectively activating M_1 on locomotion, largely due to the lack of successful characterization of M_1-selective activators. In the central nervous system, M_1 is heavily enriched in the hippocampus, neocortex, amygdala and striatum. Despite its well-established role in learning and memory, M_1 is rarely implicated in locomotion and movement control compared to its subtype family member M_2 (Gomeza et al., 1999). However, M_1 is found in the dopamine-2-(D2) expressing medium spiny neurons (MSN) in the striatum, which have projections to ventroanterior and ventrolateral thalamus (VTh) via globus pallidus (Surmeier, Ding, Day, Wang, & Shen, 2007). The VTh in turn sends projection to the primary motor cortex and is important for movement initiation (Alexander & Crutcher, 1990). Therefore, M_1 could potentially have a neuromodulatory role in movement control and locomotion. For example, hyperactivity in locomotion as been shown in M_1-knockout mice accompanied by elevated DA transmission in the striatum (Gerber et al., 2001). Moreover, place field activity is known to correlate with self-motion cues such as running speed (Terrazas et al., 2005). For example, hippocampal theta rhythm increases as a function of running speed (Sainsbury, Heynen, & Montoya, 1987). Hippocampal gamma oscillations were also shown to be altered by running speed, with gamma-band coherence shifting to higher frequencies at faster running speeds (Ahmed & Mehta, 2012). Therefore, the observed suppression in hippocampal CA3-CA1 low gamma coherence and suppression in

hippocampal CA3 spike – CA1 theta field coherence by VU0364572 in the Lebois (2014) study could both be attributed to decreases in running speeds.

Two possibilities exist: 1) VU0364572 acted directly on the M_1 receptors in the hippocampus (or adjacent areas such as the entorhinal cortex) to influence place cells; 2) VU0364572 acted indirectly on the hippocampus by impacting the locomotor activity of rats, which in turn influenced place cell activity. The activation of mAChRs has been shown to result in both excitation and inhibition of DA transmission in the basal ganglia (Raiteri, Leardi, & Marchi, 1984; Xu, Mizobe, Yamamoto, & Kato, 1989), suggesting that the modulating role of mAChRs at multiple levels of DA systems is dependent upon the level of mAChRs activation. Locomotor activity was shown to be substantially decreased in rats in pan-mAChR activation when treated with nonselective AChEI, Donepezil (Myhrer, Enger, & Aas, 2010). Hyperactivity in locomotion has been shown in M_1-knockout mice accompanied by elevated DA transmission in the striatum (Gerber et al., 2001) and following microinjection of M_1 selective antagonist (Shapovalova, Kamkina, & Mysovskii, 2005). Therefore, activating M_1 with high doses of M_1 agonist such as VU0364572 may be associated with hypoactivity in locomotion due to a decreased DA transmission modulated by M_1. Nevertheless, M_1 PAM such as BQCA has been shown to have no spontaneous locomotion side effects in a previous study (Chambon, Jatzke, Wegener, Gravius, & Danysz, 2012).

The purpose of the present study was to reanalyze the locomotion data from the Lebois (2014) study to determine the extent to which locomotor activity differed between each drug condition (10 mg/kg and 30 mg/kg of VU0364572, 30 mg/kg of BQCA or 3.0 mg/kg of Donepezil) and vehicle control. One possibility was that the medium (10

mg/kg) and high dose (30 mg/kg) of VU0364572 would significantly decrease locomotor activity of tested animals. An additional possibility was that 30 mg/kg of BQCA and 3.0 mg/kg of Donepezil would not significantly impact locomotor activities of tested animals, consistent with previous findings. These possible outcomes (decreased locomotion by M_1 activation via VU0364572) would suggest that subsequent neural analyses of this compound *in vivo* would require the inclusion of locomotion as a covariate. The alternate outcome (no differences in locomotion by M_1 activation via VU0364572) would suggest that any influence of the drugs on neural activity could not simply be accounted for by differences in locomotion.

METHODS

Subjects

Behavioral studies were conducted using young adult male F344×BNF1 rats (n = 4), weighing 300-400 grams. Subjects were individually housed under a 12 h light/dark cycle with free access to water and were mildly food deprived and trained to explore the testing enclosure prior to behavioral testing. Behavioral testing occurred during the light cycle. All procedures involving rats were approved by the Institutional Animal Care and Use Committee at Emory University.

Open Field Exploration Task

Locomotor behavioral studies were conducted in different shapes of the same "morph box" testing enclosure in a dimly lighted room. The morph box consisted of 36 serially connected black walls that could be shaped to a series of five geometric contexts (square-octagon-hexagon-circle-square) with same heights (50 cm) and same inner surface areas (63 cm × 63 cm). The morph box rested on a glass surface above a 1 m high table and was located at the center of the room. Food-restricted rats were introduced to and motivated to search the morph box for randomly scattered food rewards for 15 minutes. Following exploration in each context, rats were removed and allowed to rest on a nearby stool for 5 minutes while the shape of the morph box was changed to the next shape in the test sequence. A complete test day lasted for about 1.5 hours, and a complete test sequence for five drug conditions including vehicle control lasted for 7 days per subject.

Surgery and Data Acquisition

Stereotaxic surgery was performed after rats were deeply anesthetized with isoflurane (1–3% in oxygen) and administered buprenorphine (0.05 mg/kg) as an analgesic. Rats were implanted with a chronic recording assembly that contained independently movable tetrodes. The neural data collected from the implantation were not a part of the present analyses. However, two LED lights (a green and a red) that were used for tracking the location of rats' head were assembled to the chronic recording assembly 15 minutes prior to testing. A video camera was placed on top of the morph box to record all activities in response to LED lights within the morph box. Timestamps for all coordinates were obtained based on frames of the video files. Custom written software implemented in MATLAB was used to track the locations of the LED lights and was used to determine the head locations of rats within the morph box.

Drug Conditions

Five different drug conditions were included in the study: vehicle, 10 mg/kg VU0364572, 30 mg/kg VU0364572, 10 mg/kg BQCA and 3.0 mg/kg Donepezil. All rats received vehicle control treatments on the first and last day of testing sequence to avoid long-term effects of drugs. The vehicle treatment consists of both an injection of saline and a jello tablet containing no drug. One type of drug condition was administered per test day, and drug conditions were randomized across rats to avoid any bias due to drug order. All drugs were dosed 30 minutes prior to testing to achieve and maintain maximum potency during testing. Both VU0364572 (10 and 30 mg/kg) and Donepezil (3.0 mg/kg) were orally administered (p.o.) (using a pre-mixed jello tablet containing the

drug) because of their established oral bioavailability (Lebois et al., 2011; Sugimoto, Yamanishi, Ogura, Iimura, & Yamatsu, 1999). We should note that the orally administered doses of VU0364572 in the present study were higher than those in Digby et al. (2012) where VU0364572 was administered intraperitoneally (i.p.). All other drugs were systemically dosed. Rats were injected subcutaneously (s.c.) with BQCA at a dose of 10 mg/kg. All aforementioned drug doses were selected based on previous studies to engage memory circuitry in rats *in vivo* (Lebois et al., 2011; Mistry et al., 2013; Sugimoto et al., 1999). All rats were allowed to recover for 24 hours (~3-4 drug half-lives) following behavioral testing to prevent possible confounding drug interactions.

Data Analyses

Analysis of running speed across drug conditions

Distances travelled (in pixels) between every frame were calculated based on coordinates and timestamps acquired from head position of the rats (LED lights) in the morph box. Instantaneous running speeds were then calculated and converted to centimeters per second after applying a custom written low pass filter function (0-3 Hz) to smooth the running speed curves. The mean overall running speed was found and plotted for each drug condition across 4 rats. The mean running speed of the rats while they were locomoting was also calculated after filtering out instantaneous running speeds smaller than 35 pixels/frame (~ 3.2 cm/s) to account for all freezing, stopping and grooming behavior of rats. All data analyses were conducted using MATLAB (MathWorks) unless otherwise noted.

Analysis of locomoting vs. stationary across drug conditions

Three running speed bins were defined to correspond to observations of the videos: stationary (< 35 pixels/frame or 3.2 cm/s), medium speed (35-80 pixels/frame or 3.2-7.2 cm/s) and high speed (> 80 pixels/frame or 7.2 cm/s).

Analysis of thigmotaxic behavior across drug conditions

Thigmotaxic behavior describes the tendency of rodents to mainly explore the peripheral zones of an open field, which was proposed as an index of anxiety (Simon, Dupuis, & Costentin, 1994). Analysis of thigmotaxic behavior was conducted using the "inpolygon" function within MATLAB. Rats were considered to be showing thigmotaxic behavior if the smallest distance from their LED light coordinate to the nearest wall was less than 1/5 of the total shape width, i.e., for a square shape (63 cm × 63 cm), any coordinate with distance smaller than 13 cm to its nearest wall was considered a thigmotaxic coordinate (Figure 2A). For hexagon, octagon and circle shapes, thigmotaxic behavior was quantified according to the parameters used for hexagon, i.e., area within 13 cm from all walls of a hexagon was considered thigmotaxic (Figure 2A) due to the similarity of the wall positions for these conditions.

Statistical Analysis

One-way repeated measures analysis of variance (ANOVA) tests were used to compare the effect of drugs on locomotive parameters including mean overall running speed, mean locomotive running speed, percent of time stationary and percent of time thigmotaxic in five drug conditions (vehicle, 10 mg/kgVU0364572, 30 mg/kg

VU0364572, 10 mg/kg BQCA and 3.0 mg/kg Donepezil). All statistical tests were conducted using SPSS 22 (IBM) built-in function.

RESULTS

In general, no appreciable effects of M_1-selective drugs on the running speed were observed. Figure 3 shows the mean overall running speed for each drug conditions across 4 subjects. A one-way repeated measures ANOVA (see Methods) was conducted to compare the effect of drugs on mean running speed in the five drug conditions (vehicle, 10 mg/kg VU0364572, 30 mg/kg VU0364572, 10 mg/kg BQCA and 3.0 mg/kg Donepezil). We did not observe significant effects of any M_1-selective drugs on mean running speed versus vehicle control, $F(4, 12) = 2.151$, $p = 0.137$ (Figure 3A). When we compared the mean running speed of subjects while rats were locomoting, no effects of M_1-selective drugs were observed, $F(4, 12) = 0.496$, $p = 0.739$ (Figure 3B). Although there was no main effect of M_1-selective drugs on running speed, we asked if the effect of drugs on running speed could differ in different shapes of morph box enclosures. Specifically, we analyzed the mean running speeds of rats in square, octagon, hexagon and circle shapes independently across five drug conditions. However, we did not observe significant effects of M_1-selective drugs on mean running speed in square ($F(4, 12) = 1.735$, $p = 0.207$), octagon ($F(4, 12) = 0.866$, $p = 0.512$), hexagon ($F(4, 12) = 0.850$, $p = 0.520$), or circle shapes ($F(4, 12) = 0.952$, $p = 0.468$).

To address the possibility that M_1-selective drugs had an effect on the mean percent of time subjects spent being stationary in the morph box, we also conducted a one-way repeated measures ANOVA to compare the effect of drugs on percent of stationary time in five drug conditions. Figure 4 shows results, and statistical testing indicated that we did not observe any statistically-significant effects of M_1-selective

drugs on the mean percent of stationary time ($F(4, 12) = 0.933$, $p = 0.477$). To more easily visualize the results across drug conditions, we also included a pie chart (Figure 5) detailing the percent of time each rat spent in each speed bin (stationary < 3.2 cm/s, medium speed 3.2-7.2 cm/s, and high speed > 7.2 cm/s) in five drug conditions. According to figure 5, the percent of time each rat spent in each speed bins was consistent within subjects across five drug conditions, but we saw individual differences among subjects. Specifically, subject rat 4 spent no time running at high speed compared to the other three rats, suggesting that individual differences of locomotive behavior did exist among rats. The decreased overall running speed of rat 4 was also consistent with a noticeable difference in mean locomotive running speed of rat 4 from other three subjects (Figure 3B).

Finally, to test the possibility that M_1-selective drugs had effects on the thigmotaxic behavior of rats, we used a one-way repeated measures ANOVA to compare the effect of drugs on the mean percent of thigmotaxic time in five drug conditions. Figure 2B shows the results and statistical testing indicated that there was no significant effect of M_1-selective drugs on the thigmotaxic behavior of rats ($F(4, 12) = 0.368$, $p = 0.827$). Overall, rats spent approximately 60% of their times exploring the peripheral zones of the morph box.

DISCUSSION

The current findings show that systemically-administered M_1-selective activators, including VU0364572 and BQCA, did not significantly impact measures of locomotor activity in rats during a random foraging task. Specifically, running speeds, percent of stationary time, and thigmotaxic behavior did not statistically significantly differ between any of the drug conditions and the (vehicle) control condition. Indeed, the rat-to-rat variability was greater than the variability in these measures across drug conditions. To the extent that these locomotion measures reflected behavioral states (locomoting vs. not locomoting), arousal (running speed), or anxiety-like behavior (thigmotaxis), the results indicated that the M_1 activators did not appreciably impact these variables. This finding is important insofar as it predicts a lessor chance at observing motoric or anxiety-related side effects of these drugs in any possible future studies with humans.

This finding is also important as it relates to neural data collected in the same rats and same testing sessions as the locomotion data. Specifically, Lebois (2014) found that selective M_1 activation via VU0364572 decreased hippocampal subfield CA3-CA1 functional synchrony yet enhanced hippocampal place field representations. Meanwhile, VU0364572 did not alter locomotor states such as arousal and anxiety-like behavior in the same rats in the present study. Thus, this study served as an important counterargument to the possibility that VU0364572 indirectly influenced place cell activity in the hippocampus activity via more direct effects on locomotor behavioral states. That is, the robust effects of the M_1 activators on place cell activity reported by Lebois (2014) are unlikely to be explained by simple locomotion confounds.

One possible explanation for not observing an effect of M_1-selective activation on locomotor activity is due to the partial agonist nature of VU0364572. Although VU0364572 was first reported as an allosteric agonist for M_1 (Lebois et al., 2010), Digby et al. (2012) found that VU0364572 displayed a bitopic behavior in that it had an orthosteric partial agonist activity in systems with reduced receptor reserves or no receptor reserves. Therefore, the variable activity of VU0364572 might be dependent on receptor expression levels in different systems. For example, the aforementioned electrophysiological efficacy of VU0364572 was because of the high expression level of M_1 in the hippocampal circuitry with high M_1 reserves, whereas VU0364572 exhibited very weak efficacy in inducing excitatory effects in striatal MSNs where moderate M_1 receptor reserves exist. Since striatal MSNs project to VTh via globus pallidus, and VTh is thought to be important for movement initiation, limited excitatory effects in MSNs mediated by weak M_1 activation by VU0364572 might account for the observed no locomotive effect in the present study.

A limitation of the current study is that we utilized a morph box and head LED lights system to assess locomotor activity, which only provides us with two-dimension coordinates (X and Y axis) of rats' location from the top-down view of the testing apparatus. In the future, we could incorporate infrared light-beams on the walls of the morph box to account for the third dimension (Z axis) of rats' location as well as assessing additional locomotor measures such as jumping activity, ambulatory episodes, head directions etc. Also, since we observed high rat-to-rat variability within each drug conditions, we will expand the subject to n = 10-14 rats to account for the individual locomotive variability between subjects. Last but not least, as the promising compounds

such as VU0364572 undergo further chemical optimizations and successfully enter clinical trials, we should incorporate measures of human locomotion such as gaiting analyses, walking, running and jumping measurements into the long-term future clinical studies to assess the safety and efficacy of M_1 activators.

LIST OF FIGURES

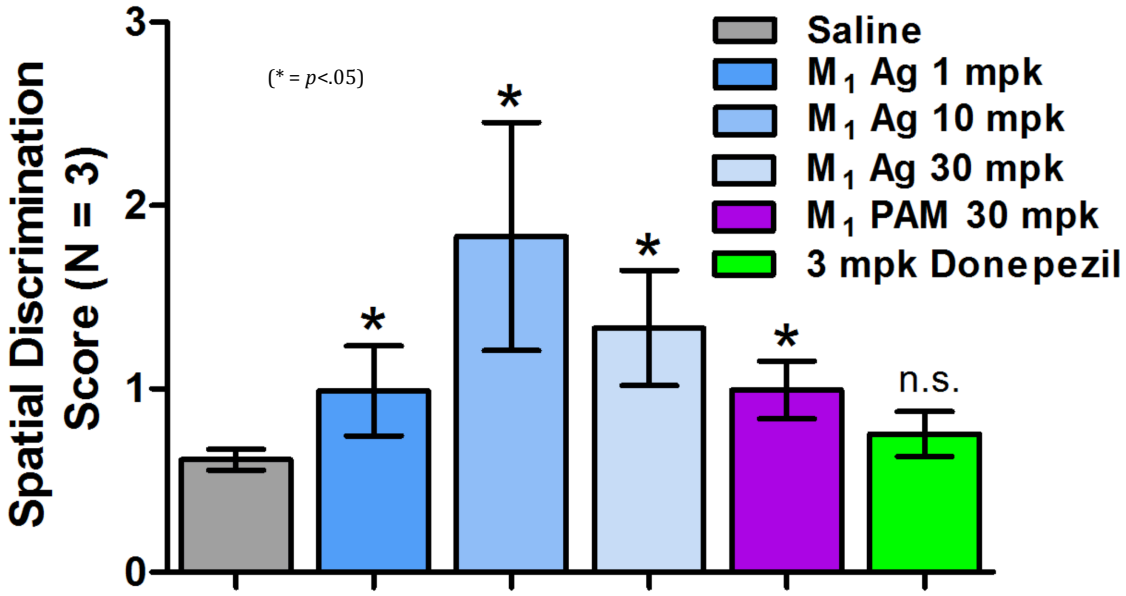

Figure 1. Average spatial discrimination score (± SEM) across six test sessions.

M_1 agonist dosing (VU0364572) improves the ability of the hippocampus to encode novel information versus a saline control and the current AD standard of care, Donepezil (AChEI). The M_1 potentiator, BQCA, also improves the ability of the hippocampus to encode novel information. Reproduced from Lebois (2014).

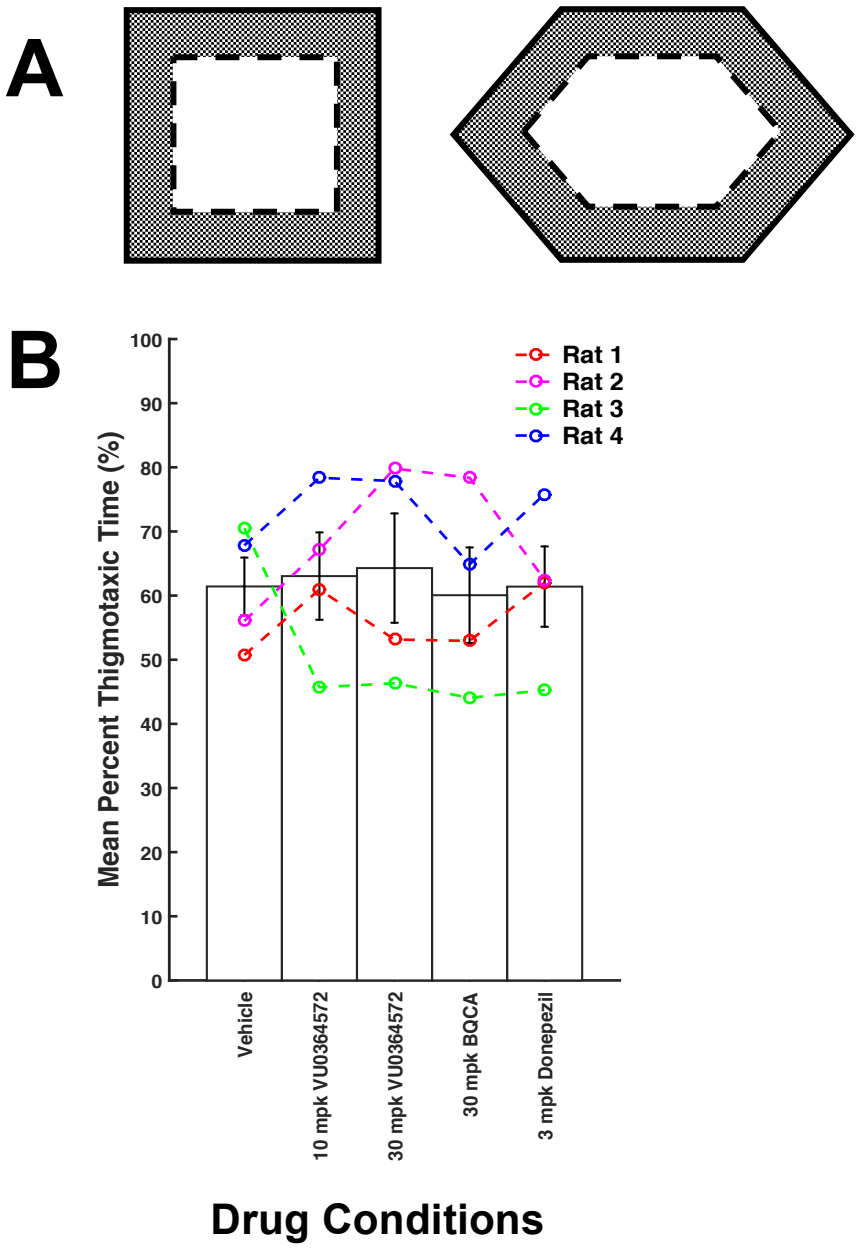

Figure 2. Thigmotaxis Analysis

A) Example thigmotaxic region (shaded) shown in a square and a hexagon.
B) Mean percent of thigmotaxic time for five drug conditions: saline vehicle, 10 mg/kg VU0364572, 30 mg/kg VU0364572, 30 mg/kg BQCA and 3.0 mg/kg Donepezil. Overall thigmotaxic behavior did not differ among the five drug conditions. Data are mean ± SEM, and each data point is from four rat subjects (individually shown in red, pink, green and blue colored lines).

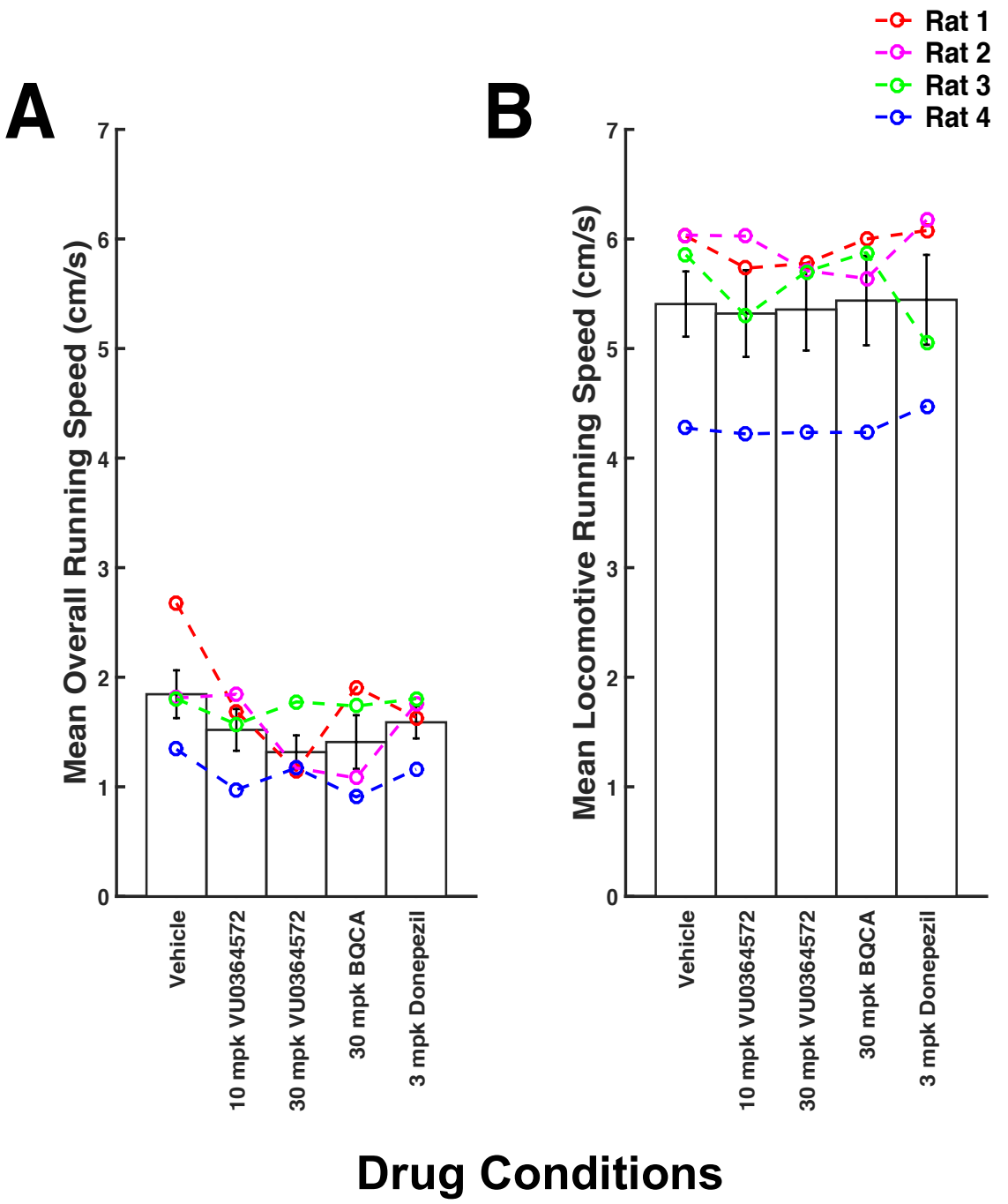

Figure 3. Running Speed Analysis

A) Mean overall running speed and B) mean locomotive running speed for five drug conditions: saline vehicle, 10 mg/kg VU0364572, 30 mg/kg VU0364572, 30 mg/kg BQCA and 3.0 mg/kg Donepezil. Overall running speed and locomotive running speed did not differ among the five drug conditions. Data are mean ± SEM, and each data point is from four rat subjects (individually shown in red, pink, green and blue colored lines).

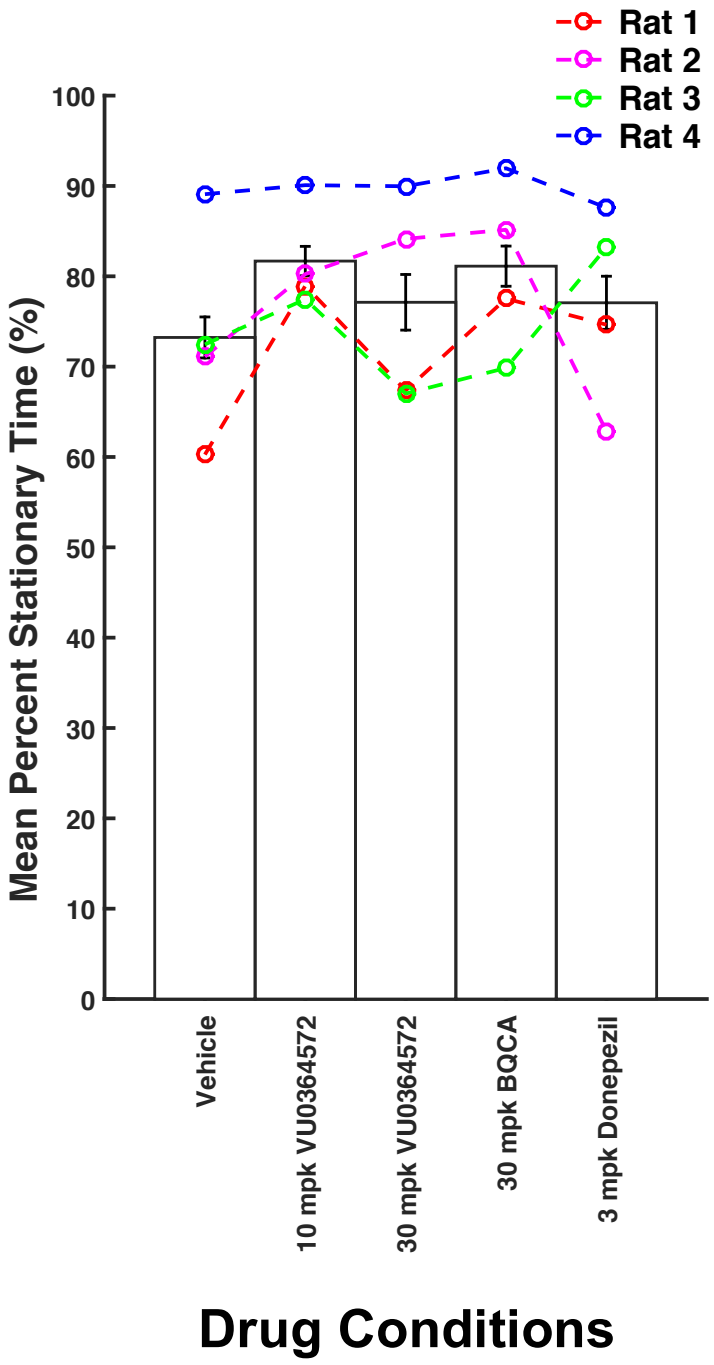

Figure 4. Percent of Time Stationary

Mean percent of stationary time for five drug conditions: saline vehicle, 10 mg/kg VU0364572, 30 mg/kg VU0364572, 30 mg/kg BQCA and 3.0 mg/kg Donepezil. The percent of time rats spent in stationary did not differ among the five drug conditions. Data are mean ± SEM, and each data point is from four rat subjects (individually shown in red, pink, green and blue colored lines).

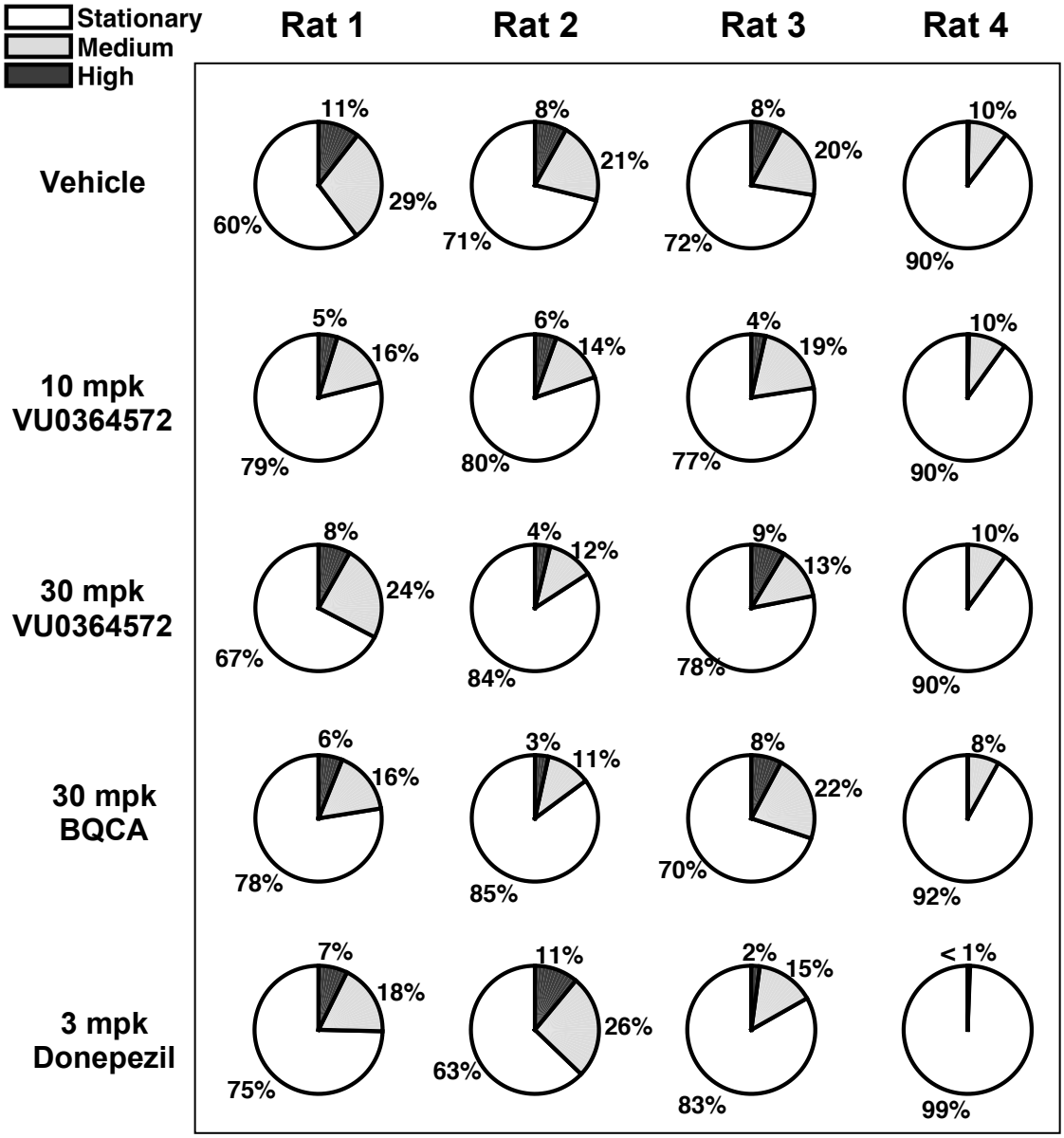

Figure 5. Percent of Time in Three Speed Bins

Percent of time each rat spent in three speed bins (stationary < 3.2 cm/s, medium speed 3.2-7.2 cm/s, and high speed > 7.2 cm/s) for five drug conditions: saline vehicle, 10 mg/kg VU0364572, 30 mg/kg VU0364572, 30 mg/kg BQCA and 3.0 mg/kg Donepezil. The percent of time rats spent in each speed bins was consistent within subjects across five drug conditions, but individual differences existed between subjects.

REFERENCES

Abe, K., Nakata, A., Mizutani, A., & Saito, H. (1994). Facilitatory but nonessential role of the muscarinic cholinergic system in the generation of long-term potentiation of population spikes in the dentate gyrus in vivo. *Neuropharmacology, 33*(7), 847-852.

Ahmed, O. J., & Mehta, M. R. (2012). Running speed alters the frequency of hippocampal gamma oscillations. *J Neurosci, 32*(21), 7373-7383.

Alexander, G. E., & Crutcher, M. D. (1990). Functional architecture of basal ganglia circuits: neural substrates of parallel processing. *Trends Neurosci, 13*(7), 266-271.

Amaral, D. G., & Kurz, J. (1985). An analysis of the origins of the cholinergic and noncholinergic septal projections to the hippocampal formation of the rat. *J Comp Neurol, 240*(1), 37-59.

Anagnostaras, S. G., Murphy, G. G., Hamilton, S. E., Mitchell, S. L., Rahnama, N. P., Nathanson, N. M., & Silva, A. J. (2003). Selective cognitive dysfunction in acetylcholine M1 muscarinic receptor mutant mice. *Nat Neurosci, 6*(1), 51-58.

Auerbach, J. M., & Segal, M. (1996). Muscarinic receptors mediating depression and long-term potentiation in rat hippocampus. *J Physiol, 492 (Pt 2)*, 479-493.

Blitzer, R. D., Gil, O., & Landau, E. M. (1990). Cholinergic stimulation enhances long-term potentiation in the CA1 region of rat hippocampus. *Neurosci Lett, 119*(2), 207-210.

Bodick, N. C., Offen, W. W., Levey, A. I., Cutler, N. R., Gauthier, S. G., Satlin, A., . . . Paul, S. M. (1997). Effects of xanomeline, a selective muscarinic receptor agonist, on cognitive function and behavioral symptoms in Alzheimer disease. *Arch Neurol, 54*(4), 465-473.

Burgard, E. C., & Sarvey, J. M. (1990). Muscarinic receptor activation facilitates the induction of long-term potentiation (LTP) in the rat dentate gyrus. *Neurosci Lett, 116*(1-2), 34-39.

Caulfield, M. P. (1993). Muscarinic receptors--characterization, coupling and function. *Pharmacol Ther, 58*(3), 319-379.

Chambon, C., Jatzke, C., Wegener, N., Gravius, A., & Danysz, W. (2012). Using cholinergic M1 receptor positive allosteric modulators to improve memory via enhancement of brain cholinergic communication. *Eur J Pharmacol, 697*(1-3), 73-80.

Colovic, M. B., Krstic, D. Z., Lazarevic-Pasti, T. D., Bondzic, A. M., & Vasic, V. M. (2013). Acetylcholinesterase inhibitors: pharmacology and toxicology. *Curr Neuropharmacol, 11*(3), 315-335.

Coyle, J. T., Price, D. L., & DeLong, M. R. (1983). Alzheimer's disease: a disorder of cortical cholinergic innervation. *Science, 219*(4589), 1184-1190.

Davies, P., & Maloney, A. J. (1976). Selective loss of central cholinergic neurons in Alzheimer's disease. *Lancet, 2*(8000), 1403.

Digby, G. J., Noetzel, M. J., Bubser, M., Utley, T. J., Walker, A. G., Byun, N. E., . . . Conn, P. J. (2012). Novel allosteric agonists of M1 muscarinic acetylcholine receptors induce brain region-specific responses that correspond with behavioral effects in animal models. *J Neurosci, 32*(25), 8532-8544.

Drever, B. D., Riedel, G., & Platt, B. (2011). The cholinergic system and hippocampal plasticity. *Behav Brain Res, 221*(2), 505-514.

Gerber, D. J., Sotnikova, T. D., Gainetdinov, R. R., Huang, S. Y., Caron, M. G., & Tonegawa, S. (2001). Hyperactivity, elevated dopaminergic transmission, and response to amphetamine in M1 muscarinic acetylcholine receptor-deficient mice. *Proc Natl Acad Sci U S A, 98*(26), 15312-15317.

Gomeza, J., Shannon, H., Kostenis, E., Felder, C., Zhang, L., Brodkin, J., . . . Wess, J. (1999). Pronounced pharmacologic deficits in M2 muscarinic acetylcholine receptor knockout mice. *Proc Natl Acad Sci U S A, 96*(4), 1692-1697.

Halliwell, J. V. (1990). Physiological mechanisms of cholinergic action in the hippocampus. *Prog Brain Res, 84*, 255-272.

Holtzman, D. M., Morris, J. C., & Goate, A. M. (2011). Alzheimer's disease: the challenge of the second century. *Sci Transl Med, 3*(77), 77sr71.

Ibach, B., & Haen, E. (2004). Acetylcholinesterase inhibition in Alzheimer's Disease. *Curr Pharm Des, 10*(3), 231-251.

Langmead, C. J., Watson, J., & Reavill, C. (2008). Muscarinic acetylcholine receptors as CNS drug targets. *Pharmacology & Therapeutics, 117*(2), 232-243.

Lebois, E. P. (2014). Discovery and Development of Selective M1 Agonists that Protect Against Alzheimer's Pathology and Bias Hippocampal Circuitry Dynamics in Rodents (Doctoral dissertation)(In press).

Lebois, E. P., Bridges, T. M., Kennedy, J. P., Xiang, Z. X., Jadhav, S. B., Yin, H. Y., . . . Lindsley, C. W. (2009). Discovery and Characterization of a Novel Subtype-Selective M1 Allosteric Agonist for the Treatment of Alzheimer's Disease. *Faseb Journal, 23*.

Lebois, E. P., Bridges, T. M., Lewis, L. M., Dawson, E. S., Kane, A. S., Xiang, Z., . . . Lindsley, C. W. (2010). Discovery and characterization of novel subtype-selective allosteric agonists for the investigation of M(1) receptor function in the central nervous system. *ACS Chem Neurosci, 1*(2), 104-121.

Lebois, E. P., Digby, G. J., Sheffler, D. J., Melancon, B. J., Tarr, J. C., Cho, H. P., . . . Lindsley, C. W. (2011). Development of a highly selective, orally bioavailable and CNS penetrant M1 agonist derived from the MLPCN probe ML071. *Bioorg Med Chem Lett, 21*(21), 6451-6455.

Levey, A. I. (1996). Muscarinic acetylcholine receptor expression in memory circuits: implications for treatment of Alzheimer disease. *Proc Natl Acad Sci U S A, 93*(24), 13541-13546.

Marino, M. J., Rouse, S. T., Levey, A. I., Potter, L. T., & Conn, P. J. (1998). Activation of the genetically defined m1 muscarinic receptor potentiates N-methyl-D-aspartate (NMDA) receptor currents in hippocampal pyramidal cells. *Proc Natl Acad Sci U S A, 95*(19), 11465-11470.

Markram, H., & Segal, M. (1990). Long-lasting facilitation of excitatory postsynaptic potentials in the rat hippocampus by acetylcholine. *J Physiol, 427*, 381-393.

McNaughton, B. L., Barnes, C. A., Gerrard, J. L., Gothard, K., Jung, M. W., Knierim, J. J., . . . Weaver, K. L. (1996). Deciphering the hippocampal polyglot: The hippocampus as a path integration system. *Journal of Experimental Biology, 199*(1), 173-185.

McNaughton, B. L., Chen, L. L., & Markus, E. J. (1991). "Dead reckoning," landmark learning, and the sense of direction: a neurophysiological and computational hypothesis. *J Cogn Neurosci, 3*(2), 190-202.

Milner, T. A., Loy, R., & Amaral, D. G. (1983). An anatomical study of the development of the septo-hippocampal projection in the rat. *Brain Res, 284*(2-3), 343-371.

Mistry, S. N., Valant, C., Sexton, P. M., Capuano, B., Christopoulos, A., & Scammells, P. J. (2013). Synthesis and pharmacological profiling of analogues of benzyl quinolone carboxylic acid (BQCA) as allosteric modulators of the M1 muscarinic receptor. *J Med Chem, 56*(12), 5151-5172.

Mufson, E. J., Bothwell, M., & Kordower, J. H. (1989). Loss of nerve growth factor receptor-containing neurons in Alzheimer's disease: a quantitative analysis across subregions of the basal forebrain. *Exp Neurol, 105*(3), 221-232.

Muller, R. U., & Kubie, J. L. (1987). The Effects of Changes in the Environment on the Spatial Firing of Hippocampal Complex-Spike Cells. *Journal of Neuroscience, 7*(7), 1951-1968.

Myhrer, T., Enger, S., & Aas, P. (2010). Behavioral side effects in rats treated with acetylcholinesterase inhibitors suggested used as prophylactics against nerve agents. *Pharmacology Biochemistry and Behavior, 95*(3), 338-343.

Raiteri, M., Leardi, R., & Marchi, M. (1984). Heterogeneity of Presynaptic Muscarinic Receptors Regulating Neurotransmitter Release in the Rat-Brain. *Journal of Pharmacology and Experimental Therapeutics, 228*(1), 209-214.

Sainsbury, R. S., Heynen, A., & Montoya, C. P. (1987). Behavioral correlates of hippocampal type 2 theta in the rat. *Physiol Behav, 39*(4), 513-519.

Samsonovich, A., & McNaughton, B. L. (1997). Path integration and cognitive mapping in a continuous attractor neural network model. *Journal of Neuroscience, 17*(15), 5900-5920.

Shapiro, R. A., Wakimoto, B. T., Subers, E. M., & Nathanson, N. M. (1989). Characterization and Functional Expression in Mammalian-Cells of Genomic and Cdna Clones Encoding a Drosophila Muscarinic Acetylcholine-Receptor. *Proc Natl Acad Sci U S A, 86*(22), 9039-9043.

Shapovalova, K. B., Kamkina, Y. V., & Mysovskii, D. A. (2005). The effects of microinjection of the selective blocker of muscarinic M1 receptors pirenzepine into the neostriatum on the motor behavior of rats. *Neurosci Behav Physiol, 35*(6), 589-594.

Shinoe, T., Matsui, M., Taketo, M. M., & Manabe, T. (2005). Modulation of synaptic plasticity by physiological activation of M1 muscarinic acetylcholine receptors in the mouse hippocampus. *J Neurosci, 25*(48), 11194-11200.

Shirey, J. K., Brady, A. E., Jones, P. J., Davis, A. A., Bridges, T. M., Kennedy, J. P., . . . Conn, P. J. (2009). A selective allosteric potentiator of the M1 muscarinic acetylcholine receptor increases activity of medial prefrontal cortical neurons and restores impairments in reversal learning. *J Neurosci, 29*(45), 14271-14286.

Simon, P., Dupuis, R., & Costentin, J. (1994). Thigmotaxis as an index of anxiety in mice. Influence of dopaminergic transmissions. *Behav Brain Res, 61*(1), 59-64.

Sugimoto, H., Yamanishi, Y., Ogura, H., Iimura, Y., & Yamatsu, K. (1999). [Discovery and development of donepezil hydrochloride for the treatment of Alzheimer's disease]. *Yakugaku Zasshi, 119*(2), 101-113.

Surmeier, D. J., Ding, J., Day, M., Wang, Z., & Shen, W. (2007). D1 and D2 dopamine-receptor modulation of striatal glutamatergic signaling in striatal medium spiny neurons. *Trends Neurosci, 30*(5), 228-235.

Terrazas, A., Krause, M., Lipa, P., Gothard, K. M., Barnes, C. A., & McNaughton, B. L. (2005). Self-motion and the hippocampal spatial metric. *Journal of Neuroscience, 25*(35), 8085-8096.

Thompson, S., Lanctot, K. L., & Herrmann, N. (2004). The benefits and risks associated with cholinesterase inhibitor therapy in Alzheimer's disease. *Expert Opin Drug Saf, 3*(5), 425-440.

Wess, J. (1996a). Molecular biology of muscarinic acetylcholine receptors. *Crit Rev Neurobiol, 10*(1), 69-99.

Wess, J. (1996b). Molecular biology of muscarinic acetylcholine receptors. *Critical Reviews in Neurobiology, 10*(1), 69-99.

Whitehouse, P. J., Price, D. L., Struble, R. G., Clark, A. W., Coyle, J. T., & Delon, M. R. (1982). Alzheimer's disease and senile dementia: loss of neurons in the basal forebrain. *Science, 215*(4537), 1237-1239.

Wilson, M. A., & McNaughton, B. L. (1993). Dynamics of the hippocampal ensemble code for space. *Science, 261*(5124), 1055-1058.

Xu, M., Mizobe, F., Yamamoto, T., & Kato, T. (1989). Differential-Effects of M1-Muscarinic and M2-Muscarinic Drugs on Striatal Dopamine Release and Metabolism in Freely Moving Rats. *Brain Research, 495*(2), 232-242.