Received: 3 November 2021 | Accepted: 21 November 2021

DOI: 10.1002/prp2.907

ORIGINAL ARTICLE

# $M_1$ muscarinic receptor activation decreases alcohol consumption via a reduction in consummatory behavior

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**Funding information**
National Health and Medical Research Council, Grant/Award Number: 1116930 and 1120576

## Abstract

Muscarinic acetylcholine receptors (mAChRs) have been shown to mediate alcohol consumption and seeking. Both $M_4$ and $M_5$ mAChRs have been highlighted as potential novel treatment targets for alcohol use disorders (AUD). Similarly, $M_1$ mAChRs are expressed throughout reward circuitry, and their signaling has been implicated in cocaine consumption. However, whether the same effects are seen for alcohol consumption, or whether natural reward intake is inadvertently impacted is still unknown. To determine the role of $M_1$ mAChRs in alcohol consumption, we tested operant self-administration of alcohol under both fixed ratio (FR3) and progressive ratio (PR3-4) schedules. Enhancing $M_1$ mAChR signaling (via the $M_1$ PAM-Agonist PF-06767832, 1 mg/kg, i.p.) reduced operant alcohol consumption on a fixed schedule but had no effect on motivation to acquire alcohol. To determine whether these actions were specific to alcohol, we examined the effects of $M_1$ enhancement on natural reward (sucrose) self-administration. Systemic administration of PF-06767832 (1 mg/kg, i.p.) also reduced operant sucrose self-administration, suggesting the actions of the $M_1$ receptor may be non-selective across drug and natural rewards. Finally, to understand whether this reduction extended to natural consummatory behaviors, we assessed home cage standard chow and water consumption. $M_1$ enhancement via systemic PF-06767832 administration reduced food and water consumption. Together our results suggest the $M_1$ PAM-agonist, PF-06767832, non-specifically reduces consummatory behaviors that are not associated with motivational strength for the reward. These data highlight the need to further characterize $M_1$ agonists, PAMs, and PAM-agonists, which may have varying degrees of utility in the treatment of neuropsychiatric disorders including AUD.

**KEYWORDS**
addiction, alcohol use disorder, allosteric modulation, consummatory behavior, muscarinic receptor

**Abbreviations:** ACh, acetylcholine; AD, Alzheimer's disease; AUD, alcohol use disorders; KO, knockout; mAChRs, muscarinic acetylcholine receptors; NHMRC, National Health and Medical Research Council; PAM, positive allosteric modulator; PD, Parkinson's disease; PPI, prepulse inhibition; RM, repeated measures.

## 1 | INTRODUCTION

Given that acetylcholine (ACh) mediates reward,[1,2] learning, memory, and other higher-order cognitive processes,[3] the cholinergic system is an appealing pathway to target for the treatment of neuropsychiatric disorders, including addiction. Recently, we have implicated both the $M_4$ and $M_5$ muscarinic acetylcholine receptors (mAChRs) in alcohol consumption and seeking.[4-7] However, little research has focused on $M_1$ mAChRs in drug and alcohol use disorders (AUD), with a primary focus surrounding cognition, including in Alzheimer's disease (AD), Parkinson's disease (PD), and schizophrenia.[8-10]

$M_1$ mAChRs are expressed in reward circuitry, including the amygdala, striatum, and hippocampus.[6,11] $M_1$ mAChR knockout (KO) mice show decreased conditioned place preference to cocaine and morphine[12]; however, this may be underpinned by corresponding deficits in a range of cognitive domains.[13-15] The $M_1$/$M_4$ mAChR preferring agonist xanomeline enhances cognitive functioning in rodent models[16] and in individuals with Alzheimer's Disease or schizophrenia.[17-19] Further, in mice, xanomeline administration reduced cocaine self-administration,[20,21] and increased the choice for food over cocaine in rats.[22] Promising findings with xanomeline have driven the continued interest in the discovery and development of novel muscarinic activators, with a shift to subtype-selective compounds as a safer and more effective treatment strategy.[23-25] These compounds can be classified into three types: (1) positive allosteric modulator (PAM) which bind a site topographically distinct from the orthosteric site (where endogenous ACh binds) and potentiate ACh signaling, but have no intrinsic activity per se; (2) agonists, which can bind to the orthosteric site, allosteric site, or bitopic (across both bindings sites) and cause direct activation of the $M_1$ receptor; (3) PAM-agonist, which act to potentiate ACh activity and can directly activate the $M_1$ receptor in the absence of an orthosteric agonist.[24,26] The advantage of a PAM is the modification of $M_1$ signaling in a more nuanced manner, preserving spatial and temporal receptor signaling, and enhancing receptor subtype selectivity. However, agonists (targeting the orthosteric or allosteric binding sites) are also being explored as they offer the benefit of action in the absence of endogenous acetylcholine, while PAMs require binding by the endogenous ligand before activity can be amplified.[24]

A recent study using a bitopic $M_1$ mAChR agonist, VU0364572,[27] showed acute systemic administration in rats reduced cocaine choice over food for a sustained period (up to 4 weeks).[28] However, whether this action is driven by cognitive or reward processes, generalizes across drug classes, or is mediated through positive allosteric modulation of ACh signaling is unknown. Another compound, PF-06767832, was developed as a selective $M_1$ compound, which shows both agonist and positive allosteric actions, classifying this compound as a PAM-agonist.[29] Here, we assessed PF-06767832 in alcohol consumption and motivation to consume alcohol using operant conditioning paradigms. To determine whether these actions were specific to alcohol, we also assessed the actions of PF-06767832 in sucrose, food (chow), and water consumption.

## 2 | MATERIALS AND METHODS

### 2.1 | Animals

Inbred male Indiana alcohol-preferring (iP) rats (~8 weeks at the start of the experiment, $N = 67$) were obtained from The Florey Institute of Neuroscience and Mental Health breeding colony. The parental stock was previously acquired from the late Professor T.K. Li (while at Indiana University). Rats were pair-housed (except for experiment 5 for which they were individually housed) and kept on a 12-h light-dark cycle (lights on 7 am–7 pm) with food (Barastoc rat and mouse) and tap water freely available. All experimentation was undertaken between 10 am and 2 pm iP rats were used in this study as they voluntarily consume high levels of alcohol to intoxication and show high predictive, face, and construct validity for the study of AUD.[30] All efforts we made to minimise animal suffering and experiments were performed in accordance with the Prevention of Cruelty to Animals Act (2004), under the guidelines of the National Health and Medical Research Council (NHMRC) Australian Code of Practice for the Care and Use of animals for Experimental Purposes (2013) and approved by The Florey Institute of Neuroscience and Mental Health Animal Ethics Committee. Animal studies were reported in compliance with the ARRIVE 2.0 guidelines.[31]

### 2.2 | Compounds

The selective $M_1$ PAM-agonist PF-06767832 (*N*-[(3R,4S) −3-Hydroxytetrahydro-2*H*-pyran-4-yl]-5-methyl-4-[4-(1,3-thiazol-4-yl)benzyl]pyridine-2-carboxamide) (Sigma-Aldrich, NSW, AUS) dissolved in 10% Tween80 (Sigma-Aldrich) in sterile saline was administered at 1 mg/kg i.p. (1 ml/kg) based on previous studies.[29]

### 2.3 | Locomotor activity apparatus

Locomotor activity (distance traveled in meters) was recorded using photobeam detectors in a 43.2 × 43.2 × 30.5 cm apparatus (Med Associates) as per our previous studies.[32,33]

### 2.4 | Home cage drinking procedure (intermittent access)

Rats received access to two bottles over three 24 h-sessions per week, one containing 20% ethanol (v/v) and one containing tap water.[33-35] Solutions were prepared by diluting alcohol in tap water from 100% (v/v) pure ethanol. Daily access was given at 10.00 am. The alcohol bottle was exchanged 24 h later with a water bottle for the subsequent 24 h period (e.g., 24 h alcohol-free). After this period, a water bottle was exchanged for a 20% alcohol bottle, and the position of the alcohol bottle was swapped from the previous session to prevent a side preference forming. The total alcohol consumption

was calculated for each session (grams), using the difference in weight from the start to the end of the session multiplied by the density of 20% ethanol (0.97) and divided by the number of rats per cage.

## 2.5 | Operant self-administration apparatus

Operant self-administration was conducted as per our previous reports.[7,35,36] Standard operant chambers (Med Associates) located in ventilated cubicles fitted with sound-proofing were used. Individual chambers were equipped with two retractable levers, located on opposite sides of the chamber. Reward delivery on the active lever resulted in 0.1 ml ethanol (10% v/v, experiment 1 & 2) or 0.1 ml sucrose (1.25%–5% w/v, experiment 3) into the receptacle controlled by a 220v syringe pump driver connecting a 50 ml syringe to an 18-gauge blunt needle via silastic tubing. Inactive lever pressing did not result in any delivery. Data were recorded by Med-PC IV software (Med Associates) connected to the chambers via a computer.

## 2.6 | Experimental design

### 2.6.1 | Experiment 1: The role of $M_1$ mAChRs in locomotor activity

To determine if PF-06767832 had any effect on locomotor activity, alcohol naïve rats ($n = 12$) were habituated to daily i.p. injections (vehicle solution, 10% Tween80 in milliQ $H_2O$). On the test day, rats were randomly assigned to receive a vehicle (1 ml/kg, i.p., $n = 6$) or PF-06767832 (1 mg/kg, i.p., $n = 6$) injection, and were directly placed into the locomotor apparatus for 1 h to test spontaneous locomotor activity.

### 2.6.2 | Experiment 2: The role of $M_1$ mAChRs in fixed ratio alcohol self-administration

Next, given the recent evidence that $M_1$ receptor enhancement reduces cocaine choice in male rats,[28] we assessed whether enhancing $M_1$ receptor activity reduced operant self-administration for alcohol in a separate cohort of rats ($n = 16$). Rats first underwent 9–12 24-h sessions of intermittent alcohol consumption in the home cage. Next, rats were trained to self-administer alcohol on a FR3 schedule (3 lever presses were required for 1 alcohol delivery) for >30 sessions each lasting 20 min. Prior to testing, rats were habituated to daily vehicle injections for 4–5 days. On the test day, rats received either PF-06767832 (1 mg/kg, i.p. $n = 8$) or vehicle (1 ml/kg, i.p. $n = 8$) and were placed back into their home cage. After 30 min, rats were placed into an operant chamber, and lever responding for 10% alcohol was assessed in a 20-min test session. After 3 days of baseline FR3 self-administration, rats undertook a second test, receiving the opposite treatment in a randomized and counterbalanced manner. Based on the sustained decrease in cocaine choice over food previously observed,[28] we also analyzed operant self-administration data from the same rats when they underwent an operant self-administration session 24 h after PF-06767832 or vehicle administration.

### 2.6.3 | Experiment 3: The role of $M_1$ mAChRs in motivation to consume alcohol

In another cohort of rats, we next assessed whether PF-06767832 reduced alcohol self-administration via a reduction in motivation to acquire and consume alcohol using a progressive ratio (PR3-4, $n = 17$). Similar to experiment 1, after home cage intermittent alcohol access (9–12 sessions), rats underwent operant self-administration sessions (>30 session, each 20 min, FR3 schedule). Rats were habituated to daily vehicle injections for 4–5 days prior to testing. On test day, rats received either PF-06767832 (1 mg/kg, i.p. $n = 9$) or vehicle (1 ml/kg, i.p. $n = 8$) and were placed back into their home cage. After 30 min, rats were placed into an operant chamber and lever responding for 10% alcohol assessed in a single 2-h test session on a PR3-4 schedule, in which 32 active lever responses are required for the 10th alcohol delivery.[37] We defined the breakpoint as the final ratio completed within the 2-h session.

### 2.6.4 | Experiment 4: The role of $M_1$ mAChRs in sucrose self-administration

To test whether PF-06767832 reduced natural reward consumption, rats ($n = 8$) underwent self-administration of sucrose (1.25–5% w/v) on a FR3 schedule.[5] After stabilization (10 sessions, 20 min), rats received either PF-06767832 (1 mg/kg, i.p. $n = 4$) or vehicle (1 ml/kg, i.p. $n = 4$) and were placed back into their home cage. After 30 min, rats were placed into an operant chamber and lever responding for sucrose assessed in a 20 min test session. After 3 days of baseline FR3 sucrose self-administration, rats undertook a second test, receiving the opposite treatment in a randomized and counterbalanced manner. Of note, these rats had been previously trained for alcohol self-administration but had not undergone any pharmacological experimentation. Rats were given at least 2 weeks break between alcohol and sucrose self-administration training.

### 2.6.5 | Experiment 5: The role of $M_1$ mAChRs in home cage food and water consumption

Single housed rats received either PF-06767832 (1 mg/kg, i.p. $n = 7$) or vehicle ($n = 7$) and were placed back into their home cage. After 30 min food (standard chow) and water were provided to rats and their consumption was measured 2 and 24 h later to examine any effect on general home cage consummatory behaviors as per our previous studies.[7,33] Rats were given 3 days rest before testing with the counterbalanced treatment. These rats had previously been trained for alcohol self-administration and undergone experimentation (not related to the current study). Rats were given at least 2 weeks break after prior testing before examining food and water intake.

### 2.7 | Statistical analysis

GraphPad Prism 9 statistical software was used for analysis. Locomotor timecourse data were analyzed by repeated measures (RM) two-way ANOVA (time × treatment) and total distance by unpaired student's *t*-test. For alcohol and sucrose self-administration, operant self-administration data were analyzed by RM two-way ANOVA (lever × treatment) followed by Bonferroni *post hoc* analyses. Progressive ratio breakpoint and total distance in the locomotor test were analyzed by unpaired Student's *t*-test. All operant timecourse data were analyzed by RM two-way ANOVA (time × treatment) with Bonferroni post hoc analysis and latency to first lever press analyzed by paired or unpaired Student's *t*-test. Previous studies examining similar behaviors were used to determine sample sizes. All data are expressed as mean ± SEM.

## 3 | RESULTS

### 3.1 | Experiment 1: PF-06767832 does not alter locomotor activity

First, we established whether systemically administered PF-06767832 (1 mg/kg i.p.) altered spontaneous locomotor activity (Figure 1A). Rats were tested in a 1-h locomotor test; PF-06767832 did not alter locomotor activity across time [two-way ANOVA: main effect of time $F_{(11,110)} = 59.23$, $p < .0001$, no effect of treatment $F_{(1,10)} = 0.6532$, $p = .438$, no time × treatment interaction $F_{(11,110)} = 1.356$, $p = .203$] (Figure 1B), or total locomotor activity [unpaired *t*-test: $t_{(10)} = 0.808$, $p = .438$] (Figure 1C).

### 3.2 | Experiment 2: PF-06767832 decreases alcohol self-administration

Given PF-06767832 (1 mg/kg) did not alter locomotor activity, we next sought to examine the functional role of $M_1$ mAChR in alcohol consumption by assessing whether systemic PF-06767832 administration reduced operant self-administration under a fixed ratio schedule (Figure 2A). During the last 10 days of training rats averaged 113 ± 6 active lever and 1.6 ± 0.2 inactive lever responses. Two-way ANOVA revealed a main effect of lever ($F_{(1,15)} = 149.3$, $p < .0001$), treatment ($F_{(1,15)} = 6.218$, $p < .05$) and treatment × lever interaction ($F_{(1,15)} = 6.012$, $p < .05$). Additional Bonferroni *post hoc* analysis revealed a difference in active lever ($p = .0067$), but not inactive lever ($p > .999$) responding following PF-06767832 administration (Figure 2B). Additional analysis of lever pressing across time [two-way ANOVA: main effect of time $F_{(3,45)} = 31.09$, $p < .0001$, treatment $F_{(1,15)} = 10.65$, $p < .01$ but no treatment × time interaction $F_{(3,45)} = 0.3778$, $p = .7694$. Bonferroni post hoc analysis revealed a significant difference in lever responding at 10 min ($p < .05$), with rats administered PF-06767832 showing reduced active lever responding (Figure 2C). Paired *t*-test of latency to first active lever press showed no difference between treatment groups [$t_{(15)} = 1.271$ $p = .223$] (Figure 2D). Based on previous findings, which showed a sustained decrease in cocaine choice over food following treatment with the selective $M_1$ bitopic agonist VU0364572,[28] we examined subsequent operant alcohol responding the day after PF-06767832 administration. No differences in operant alcohol self-administration were observed 24 h following PF-06767832 or vehicle administration [two-way ANOVA revealed a main effect of lever ($F_{(1,15)} = 134.1$, $p < .0001$), but no effect of treatment ($F_{(1,15)} = 0.0168$, $p = .898$) or treatment × lever interaction ($F_{(1,15)} = 0.040$, $p = .8436$) (Figure 2E).

### 3.3 | Experiment 3: PF-06767832 does not alter progressive ratio responding for alcohol

To determine whether the reduction in operant alcohol responding was driven by altering motivation to obtain alcohol, we next examined the effect of systemic PF-06767832 administration on lever pressing under a progressive ratio schedule (PR3-4, Figure 3A). During the last 10 days of training rats averaged 90 ± 4 active lever and 2.3 ± 0.3 inactive lever responses. $M_1$ mAChR enhancement

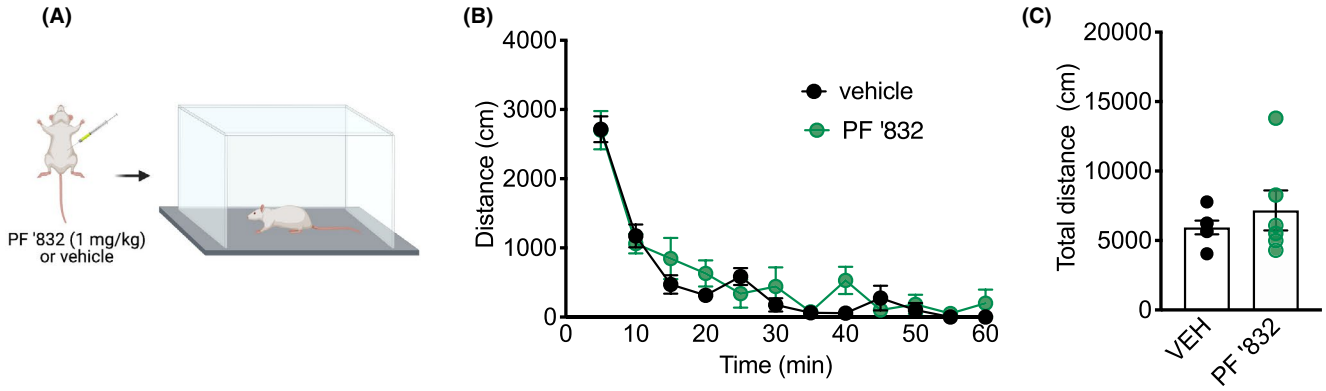


FIGURE 1 PF-06767832 does not alter locomotor activity. (A) Schematic of experimental timeline. PF-06767832 (PF '832; 1 mg/kg i.p) administered directly prior to a 1-h locomotor test does not alter (B) distance traveled across time or (C) total distance traveled. Data expressed as mean ± SEM, $n = 6$/group. Created with BioRender.com

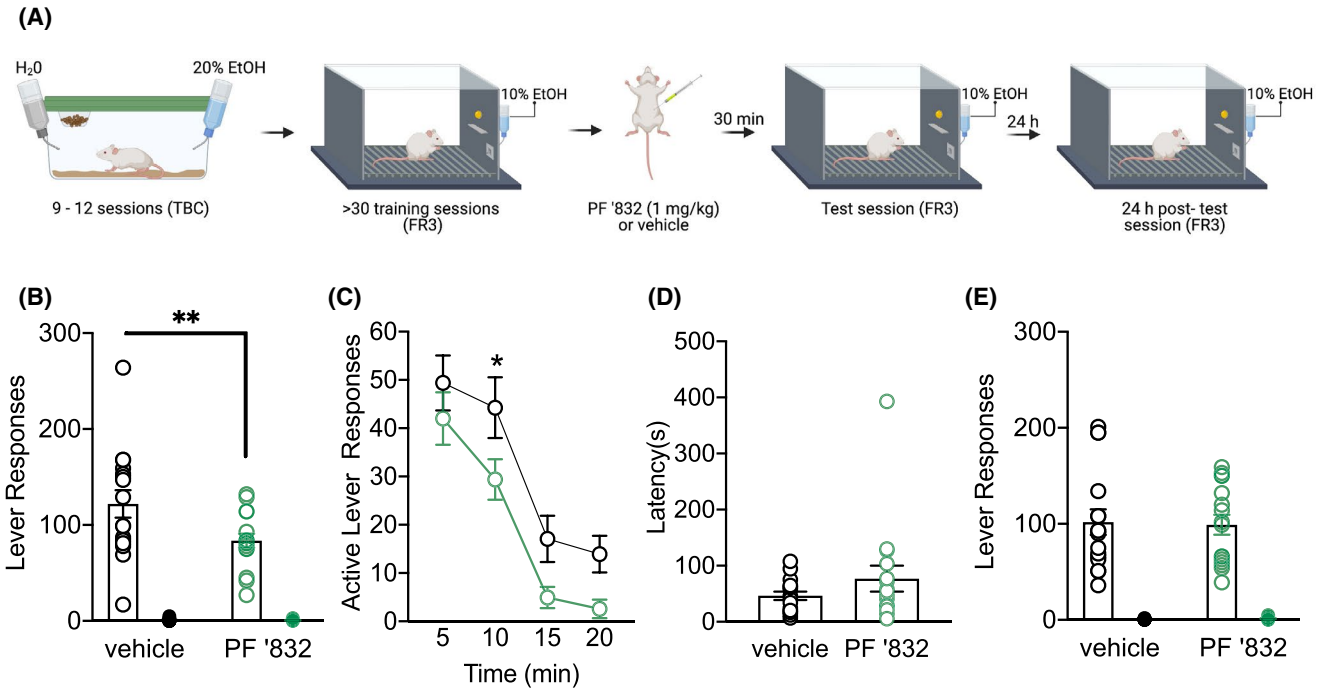


**FIGURE 2** PF-06767832 reduces alcohol self-administration. (A) Schematic of experimental timeline. PF-06767832 (PF '832; 1 mg/kg i.p) (B) decreases alcohol self-administration on the active, but not an inactive lever. (C) Timecourse reveals a decrease at the 10 min time point. (D) No difference in latency to first lever press was observed in rats that received PF'832. (E) The reduction in alcohol self-administration induced by PF '832 was not observed 24 h following administration. Data expressed as mean ± SEM, $n = 16$. TBC: two-bottle choice; FR3: fixed ratio 3. Open circle = active lever, closed circle = inactive lever. Created with BioRender.com

with PF-06767832 did not alter breakpoint on the PR3-4 schedule [unpaired *t*-test: $t_{(15)} = 0.804$, $p = .4139$] (Figure 3B). Further, no difference in lever responding was observed over the 2-hour session [two-way ANOVA: main effect of time $F_{(3,45)} = 36.90$ $p < .0001$, no effect of treatment $F_{(1,15)} = 0.3168$ $p = .5819$ or time × treatment interaction $F_{(3,45)} = 0.3070$ $p = .802$] (Figure 3C). PF-06767832 did, however, increase latency to first active lever response from an average of 17 ± 5 to 67 ± 12 s [unpaired *t*-test: $t_{(15)} = 3.864$, $p < .01$] (Figure 3D).

## 3.4 | Experiment 4: PF-06767832 decreases sucrose self-administration

To determine whether PF-06767832 reduction in alcohol consumption was specific to alcohol, or generalized to natural reward consumption, we next tested an effect on operant sucrose self-administration under FR3 (Figure 4A). During training rats averaged 155 ± 28 active lever and 1.5 ± 0.1 inactive lever presses. Administration of PF-06767832 (1 mg/kg, i.p.) reduced sucrose self-administration [two-way ANOVA: main effect of Lever $F_{(1,7)} = 109.1$, $p < .0001$, treatment $F_{(1,7)} = 7.755$, $p = .027$ and treatment × lever interaction $F_{(1,7)} = 8.697$, $p = .021$] (Figure 4B). A significant main effect of time ($F_{(3,21)} = 14.94$, $p < .0001$) and treatment were observed ($F_{(1,7)} = 8.28$, $p = .024$), but no treatment × time interaction ($F_{(3,21)} = 0.2980$, $p = .826$; Figure 4C), nor any difference in latency to first active lever press (paired *t*-test: $t_{(7)} = 0.787$, $p = .4572$) (Figure 4D).

## 3.5 | Experiment 5: PF-06767832 decreases home cage food and water consumption

Finally, based on our observations that PF-06767832 reduced operant responding for both alcohol and sucrose, we next assessed whether it impacted natural consummatory behavior (standard chow and water) in the home cage. PF-06767832 (1 mg/kg i.p.) decreased food consumption at 2 h [paired *t*-test: $t_{(13)} = 4.485$, $p < .001$] (Figure 5A), and 24 h [paired *t*-test: $t_{(13)} = 3.611$, $p < .01$] (Figure 5B). PF-06767832 also reduced water consumption at 2 h [paired *t*-test: $t_{(13)} = 4.036$, $p < .01$] (Figure 5C), but this effect did not persist at the 24 h time point [paired *t*-test: $t_{(13)} = 1.615$, $p = .130$] (Figure 5D).

## 3.6 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY,[38] and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20.[39]

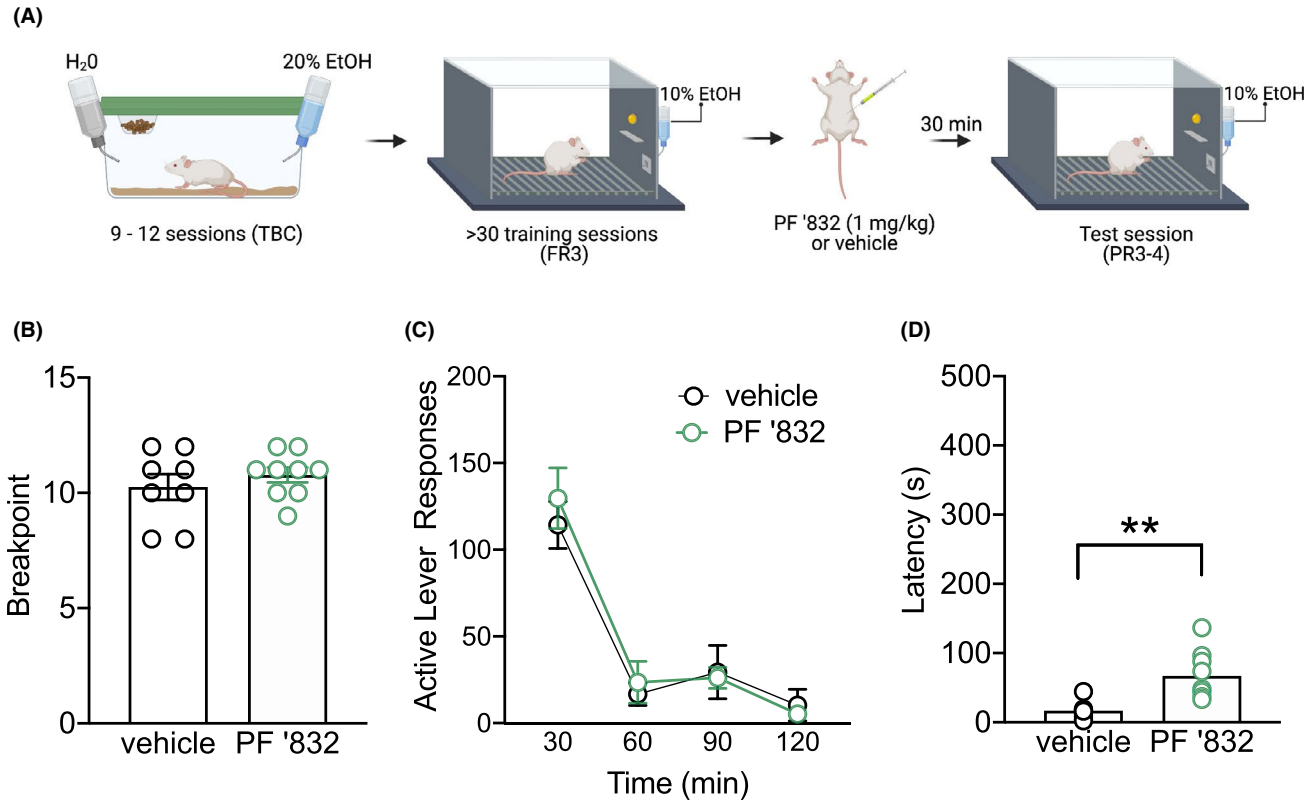


FIGURE 3 PF-06767832 does not alter motivation for alcohol. (A) Schematic of experimental timeline. PF-06767832 (PF '832; 1 mg/kg i.p) did not alter (B) breakpoint on the PR3-4 schedule, nor (C) lever pressing across time. (D) PF'832 increased latency to first active lever press. Data expressed as mean ± SEM, $n$ = 8–9/group. TBC: two-bottle choice; FR3: fixed ratio 3. Open circle = active lever, closed circle = inactive lever. Created with BioRender.com

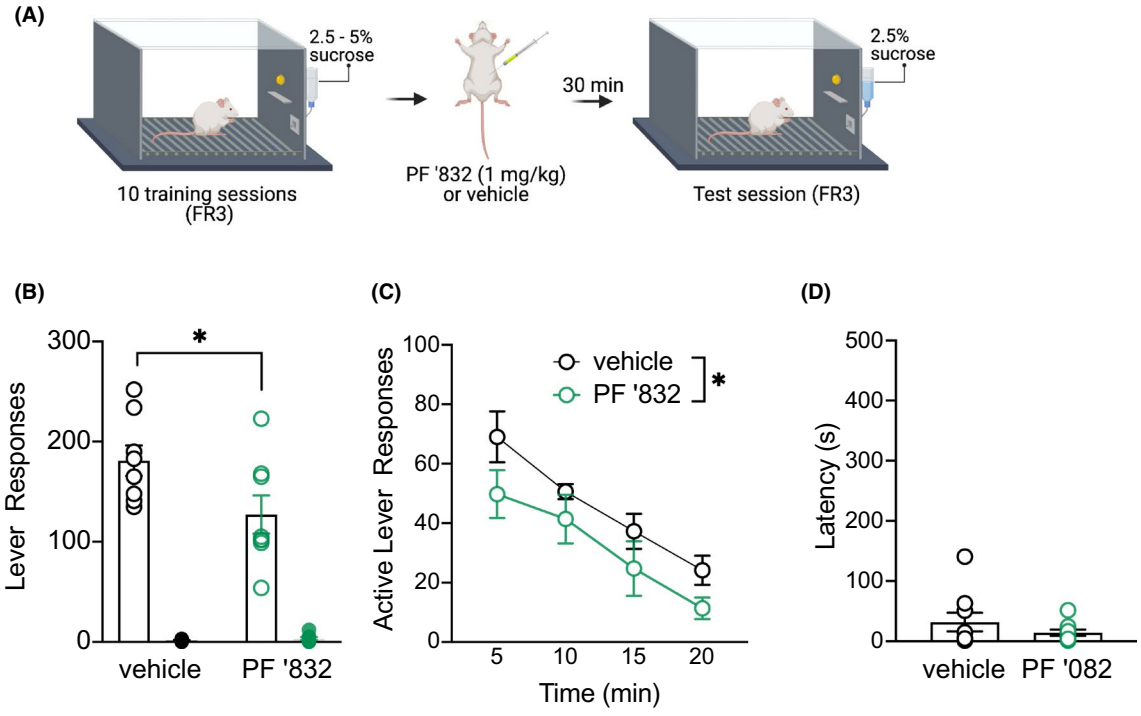


FIGURE 4 PF-06767832 reduces sucrose self-administration. (A) Schematic of experimental timeline. PF-06767832 (PF '832; 1 mg/kg i.p) reduces (B) sucrose self-administration, (C) with a difference in treatment groups observed across timecourse. (D) No difference in latency to first lever press was observed in rats which received the $M_1$ PAM. Data expressed as mean ± SEM, $n$ = 8. FR3: fixed ratio 3. Open circle = active lever, closed circle = inactive lever. Created with BioRender.com

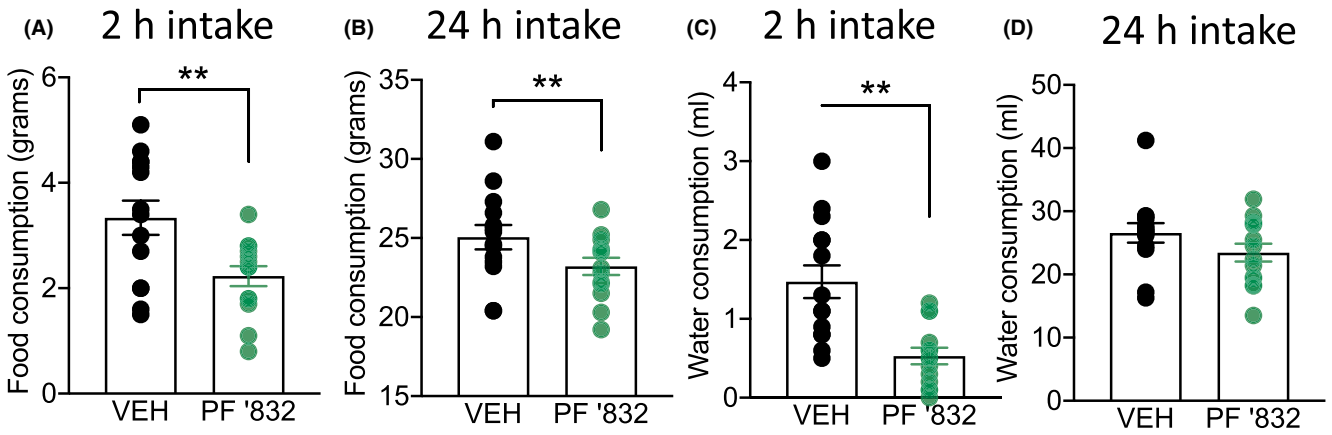


FIGURE 5 PF-06767832 reduces home cage food and water consumption. PF-06767832 (PF '832; 1 mg/kg i.p) decreases food consumption at (A) 2- and (B) 24-h timepoints and decreases water consumption at (C) 2-, but not (D) 24-h. Data expressed as mean ± SEM, $n = 14$

## 4 | DISCUSSION

Here, we provide evidence that systemic administration of the $M_1$ PAM-agonist PF-06767832 reduces consummatory behavior in male iP rats across both caloric and non-caloric solutions. We observed that $M_1$ enhancement, at a dose that did not alter locomotor activity, reduced operant alcohol self-administration on a FR3 schedule. This is in line with previous studies showing involvement of the $M_1$ receptor in other drug consumption.[12,28] However, our subsequent experiments also revealed that PF-06767832 reduced self-administration of natural reward (sucrose), suggesting the reduction in alcohol consumption is likely due to a general action of PF-6767832 in reducing all consummatory behaviors. Given these data, and the known role of the $M_1$ mAChR in cognitive domains[40,41] we next sought to determine whether our results related to interference with an instrumental process or generalized to task-free consummatory behaviors (standard show and water within the home cage environment). PF-06767832 treated rats showed reduced intake of both food and water 2 h after systemic administration, which persisted for 24 h in the case of food (but not water) consumption. Previous studies showed that fasting increased $M_1$ mAChR expression in the frontal cortex and hippocampus, but not the amygdala of mice, which was decreased after food intake,[42] supporting a potential role for $M_1$ enhancement to modulate food consumption. Interestingly, PF-06767832 at higher doses (10–45 mg/kg) can increase food intake and weight gain in rats and dogs[29] and the bitopic $M_1$ agonist VU0364572 increased food choice over cocaine.[28] $M_1$ receptors are also expressed on salivary glands where they modulate the secretion of saliva by acinar cells.[43] It is, therefore, possible that differential effects of dose or different ligand classes on "food intake" may be indirect and related to differential effects on salivation. Overall, these results suggest a potential dose-dependent action of $M_1$ modulation in relation to food consumption.

Our observed reduction in operant alcohol self-administration was transient, with no difference in alcohol consumption noted when animals were returned to the operant chamber 24 h after treatment. This result differs from recent findings using the $M_1$ agonist, VU0364572, which induced a prolonged reduction in cocaine choice over food for up to 4 weeks, accompanied by reductions in dopamine and glutamate outflow after administration.[28] The transient actions of PF-06767832 (PAM-agonist), compared with VU0364572 (bitopic agonist) may be driven by differences that exist between studies (e.g., rodent strain, drug class, and experimental paradigm). Indeed, experimental procedures employed would elicit differential cognitive requirements on the animals. The choice paradigms involve a second-order schedule, whereby an observer lever must be pressed to allow the animal to choose between the cocaine-paired or food-paired lever.[22,28] This task requires higher-order cognitive processing, while experiments in our study required lesser (first-order operant conditioning) or very little (home cage consumption) cognitive demand. Several selective orthosteric agonists have also shown that $M_1$ stimulation enhances behavioral flexibility and pro-cognitive effects.[40,44,45] Therefore, VU0364572 may reduce cocaine choice over food through altering cognitive processing that also relates to the lasting effect. Another distinct possibility is that VU0364572 may gradually reduce the reinforcing effect of cocaine, but not food, over time. We did not empirically test the possibility of such a profile developing with time in the case of PF-06767832 because our acute data showed no differential between alcohol versus sucrose. Another factor to consider when comparing our alcohol data with prior cocaine data is that alcohol has a caloric value in addition to being rewarding and $M_1$ signaling may also modulate energy intake. In agreement with this notion is the finding that the $M_1$ antagonist, biperiden, increased breakpoint for a milkshake in mice, although on an FR5 schedule did not alter consumption.[46] Nevertheless, cholinergic signaling has been linked to modulating satiety at the level of the nucleus accumbens (for review see[47]).

Another major difference in our study compared to previous findings is the class of $M_1$ compounds employed. Our study used a selective PAM-agonist, PF-06767832, which shows good oral bioavailability and robust in vivo activity.[29] In contrast, bitopic $M_1$ agonists, which span both orthosteric and allosteric binding

sites, may lack some receptor selectivity and are thought to cause a greater incidence of adverse incidences. For example, the bitopic $M_1$ agonist GSK1034702 has shown pro-cognitive effects in rodents[40]; however, adverse gastrointestinal effects of this molecule were seen in clinical trials.[48] Previous studies have suggested drug candidates that have high $M_1$ receptor selectivity, and low levels of intrinsic agonist activity (e.g., selective PAMs) provide the most potential as compounds for AD and other diseases.[49] In contrast, allosteric modulation only occurs in the presence of the endogenous ligand, maintaining the natural spatiotemporal scale and allowing nuanced manipulation of ACh signaling.[24] However, one possibility is $M_1$ PAM-agonists, which have both agonist and allosteric actions may produce similar adverse effects as traditional orthosteric agonists through over-activation of the $M_1$ receptor.[50,51] Indeed, $M_1$ PAMs lacking agonist activity are thought to provide a more optimal profile for enhancing higher-order processing.[25,50]

## 4.1 | Limitations

While our current data suggest the $M_1$ PAM-agonist PF-06767832 reduces alcohol and natural consummatory behaviors, several limitations exist that should be addressed in future work. A major limitation of our study is the solitary examination of PF-06767832; a direct comparison of $M_1$ agonist, PAM-agonist, and PAM compounds should be conducted in the future to understand differential contributions to cognitive effects, drug consumption/seeking, and side effects. Such a study should also incorporate a longitudinal testing component to examine persistence and/or the emergence of effects. Another limitation of our study is the lack of a dose-response curve. PF-06767832 has been administered up to 30 mg/kg in rats and 45 mg/kg in dogs. In dogs, doses ranging from 3 to 15 mg/kg showed gastrointestinal side effects, while 45 mg/kg also resulted in ataxia and convulsions, however, no adverse gastrointestinal side effects were observed at 1 mg/kg.[29] In rats, 1 mg/kg was sufficient to reduce amphetamine-induced locomotor activity and amphetamine-disrupted prepulse inhibition (PPI), an effect which was not observed at a lower dose (0.32 mg/kg).[29] Further, the reductions observed across fluids/food observed within our study were of similar magnitudes, with 2 h water showing the greatest magnitude in reduction (64%) following PF-06767832 administration, followed by food (33%), alcohol (30%), and sucrose (30%). Together this suggests lower doses of PF-06767832 would likely act similarly across all consummatory behaviors we tested. Additionally, in the current study, we only assessed male rats. This is a limitation given the rising prevalence of AUD in women[52] and the potential roles of sex hormones in drug and alcohol consumption.[53-55] PF-06767832 showed similar dose-dependent side effects in both male and female subjects, suggesting no overt differences in the pharmacology between sexes,[29] however, this should be empirically addressed in the future studies. Finally, little is known about the muscarinic system in Indiana alcohol-preferring (iP) rats. We have previously shown that allosteric modulation of the $M_4$ and $M_5$ receptors specifically reduces alcohol consumption in male iP rats.[4,5,7] Further, we show similar striatal $M_4$ mAChR dysregulation across human and rodent species following chronic alcohol consumption.[5] Whether the same alcohol-induced dysregulation is observed in outbred strains has not been thoroughly examined, however, iP rats are a strain that voluntarily consumes enough alcohol to achieve intoxication and dependence.[30,56]

## 4.2 | Conclusions

Collectively, our data show a non-specific effect for PF-06767832 on consummatory behavior, without altering motivation or locomotor activity. Our data highlight potential idiosyncrasies with $M_1$ muscarinic compounds, whereby agonist versus PAM versus PAM-agonist may have different actions on drug and alcohol consumption, and/or cognitive demand in different behavioral processes that may influence the ability of $M_1$ compounds to modulate drug/alcohol consumption. Understanding the selectivity and pharmacokinetics/dynamics of specific $M_1$ agonists, PAM-agonists and PAMs is required to further understand their utility for clinical development to treat alcohol and drug use disorders.

### ACKNOWLEDGMENTS

This research was supported by a National Health and Medical Research Council project grant (Grant No. 1120576 [to AJL and CJL]), of which AJL is a principal research fellow (Grant No. 1116930).

### DISCLOSURE

Authors report no biomedical financial interests or potential conflicts of interest.

### AUTHOR CONTRIBUTIONS

The study was conceived, planned, and supervised by LCW, CJL, and AJL. LCW, EJC, KLH, and NAC performed experiments and analyzed data. The manuscript was written by LCW and AJL (original draft) with input from CJL. All authors approved the manuscript prior to submission.

### DATA AVAILABILITY STATEMENT

The data from this study are available from the corresponding authors upon reasonable request.

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**How to cite this article:** Walker LC, Campbell EJ, Huckstep KL, Chen NA, Langmead CJ, Lawrence AJ. $M_1$ muscarinic receptor activation decreases alcohol consumption via a reduction in consummatory behavior. *Pharmacol Res Perspect*. 2022;10:e00907. doi:10.1002/prp2.907