LYDopaminePaper\_v4

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# Results

## Experiment 1: LY354740 impaired spatial working memory in rats

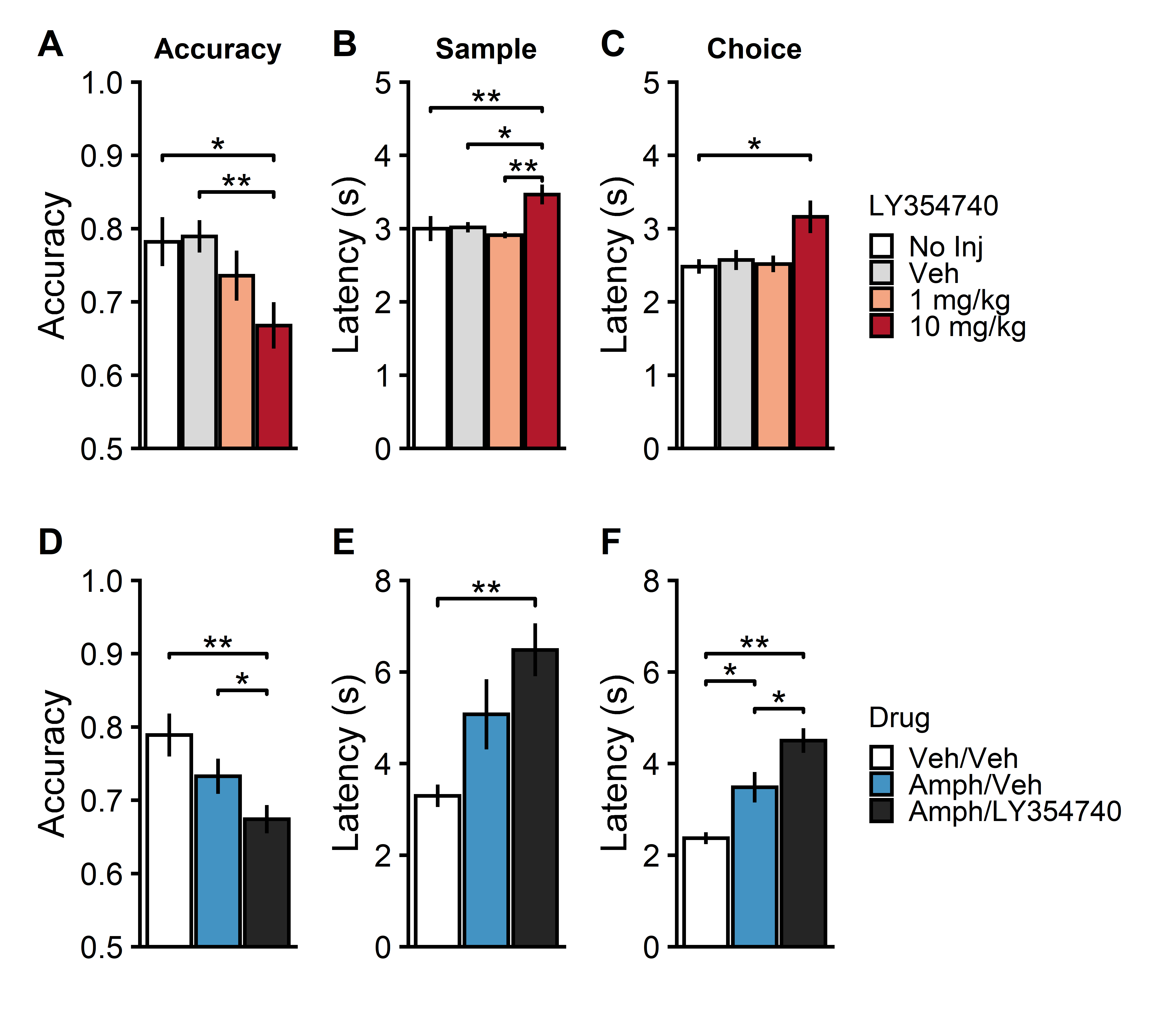
We first established an active dose of the group II metabotropic agonist in our testing environment. Rats were injected with LY354740 intraperitoneally (i.p.) at a dose of either 1 mg/kg or 10 mg/kg, 45-50 min before behavioural testing. Two control conditions consisted of a no injection (No Inj) control and a distilled water (Veh) injected condition (1 ml/kg). Rewarded alternation a T-maze was used to assess spatial working memory, and was conducted as a within-subject design.

In agreement with previous reports [REF], an i.p. dose of 10 mg/kg LY354740 decreased spatial working memory performance (Fig. 1A-C; main effect of Drug on Accuracy , , Sample Latency , , Choice Latency , ). This dose significantly reduced accuracy (Fig. 1A; No Inj vs 10 mg/kg , , Veh vs 10 mg/kg , ), and an increased latencies during the sample (Fig. 1B; No Inj vs 10 mg/kg , , Veh vs 10 mg/kg , , 1 vs 10 mg/kg ) and choice phases (Fig. 1C; No Inj vs 10 mg/kg , ).

## Experiment 2: LY354740 enhanced the effects of amphetamine on spatial working memory

Having established an active dose of LY354740, we next investigated whether the metabotropic agonist could rescue spatial working memory deficits induced by amphetamine [REF]. The same cohort of rats tested on the T-maze in experiment 1 was used in this rewarded alternation experiment with amphetamine. Rats received two consecutive i.p. injections 45-50 min before behavioural testing. They received either Veh/Veh, Amph (2.5 mg/kg)/Veh, or Amph (2.5mg/kg)/LY354740 (10 mg/kg) in a within-subjects design. *One rat had to be excluded because it failed to run on the maze in the LY354740/AMPH condition.*

Overall, drug injections significantly disrupted all three performance measures on the spatial working memory task (Fig. 1D-F; main effect of Drug on Accuracy , , Sample Latency , , Choice Latency , ). Compared to vehicle injections, amphetamine disrupted performance on the spatial working memory task, surprisingly given previous findings [REF] (Aultman and Moghaddam, 2001) this effect was only statistically robust for response latencies during the choice period (Veh/Veh vs Amph/veh: Accuracy , ), Sample Latency , , Choice Latency , ). This deficit, at least in part, reflected an increase in undirected exploratory behaviours (e.g. sniffing and rearing) in the start arm, at the expense of performing the appetitively motivated memory task. However, rather than ameliorating these effects of amphetamine as might have been expected based on previous studies [REF], combining LY354740 and amphetamine significantly impaired accuracy (Fig. 1D; Veh/Veh vs Amph/LY354740 , , Amph/Veh vs Amph/LY354740 , ), and increased response latencies during both the sample (Fig. 1E; Veh/Veh vs Amph/LY354740 , , Amph/Veh vs Amph/LY354740 , ) and choice periods (Fig. 1F;Veh/Veh vs Amph/LY354740 , , Amph/Veh vs Amph/LY354740 , ). Thus, rather than ameliorating the effects of amphetamine as predicted, LY354740 actually potentiated the effects of the dopaminergic psychomimetic during testing on the T-maze.



**Figure 1**. The effect of LY354740 and amphetamine on spatial working memory in rewarded alternation T-maze assay. Experiment 1 **(A-C)** compared the effects of no injection (No Inj), distilled water (Veh), 1 mg/kg LY354740, and 10 mg/kg LY354740 injected 45-50 mins prior to testing (within-subjects design). Experiment 2 **(D-F)** compared the effects of 2.5 mg/kg amphetamine alone (Amph/Veh) or in combination with 10 mg/kg of LY354740 (Amph/LY354740) injected 45-50 mins prior to testing (within-subjects design). The same cohort of rats was used in Experiment 1 and Experiment 2. **(A,D)** Performance accuracy measured as the proportion of trials with a correct side alternation from sample to choice. Latency (s) to enter the left or right arm of the T-maze during the sample **(B,E)** and choice **(C,F)** periods. Error bars represent +/- SEM. Statitsical significance of post-hoc simple effects (Tukey corrected) following a significant main effect of Drug: \* = *p* < .05, \*\* = *p* < .001.

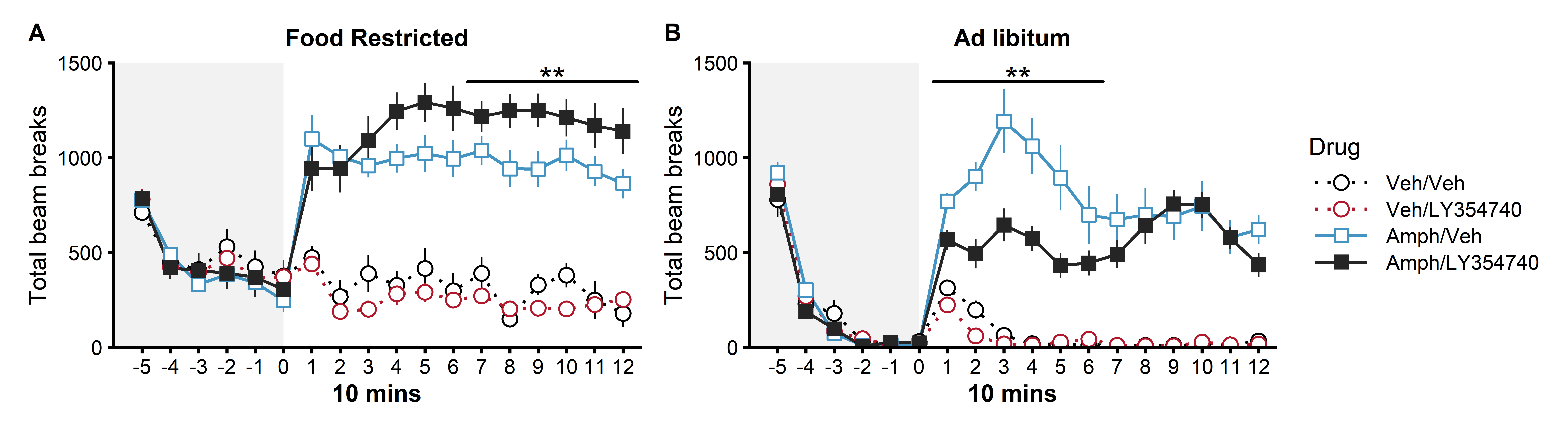
## Experiment 3: LY354740 enhanced amphetamine-induced hyperlocomotion in food restricted rats

Numerous previous studies have shown that group II metabotropic mGluR agonists, including LY354740, reduce the locomotor hyperactivity induced by amphetamine (Cartmell et al., 1999, Kim and Vezina, 2002, Galici et al., 2005, Rorick-Kehn et al., 2007, Woolley et al., 2008, Pehrson and Moghaddam, 2010). Given our unexpected findings on the T-maze, with LY354740 appearing to exacerbate the effects of amphetamine, we next assessed the effects of the mGluR agonist on amphetamine-induced hyperactivity in locomotor activity cages, in these same animals while still on food restriction. As expected, amphetamine (2.5 mg/kg) significantly increased locomotor activity (main effect of Amph , , Amph x Time , ). Surprsingly, LY354740 (10 mg/kg) significantly enhanced this amphetamine-induced hyperactivity over time rather than reduce it (Amph x LY354740 x Time , , Amph x LY354740 , ). Specifically, LY354740 significantly increased Amph induced hyperactivity 61-120 mins post injection (Amph/LY354740 vs Amph/Veh , ) but not 1-60 mins post injection (, ). In contrast, LY354740 alone did not affect locomotor activity (Veh/LY354740 vs Veh/Veh, 1-60 mins , , 61-120 mins , ). This enhancement is the effect of amphetamine is consistent with the effects on running latencies on the T-maze (Experiment 2, Fig. 1B), but at odds with the previously published results.

## Experiment 4: LY354740 reduced amphetamine-induced hyperlocomotion in ad libitum fed rats

Given these surprising results, we then re-assessed the effects of LY354740 on amphetamine-induced locomotor hyperactivity in a separate cohort of experimentally naïve rats maintained with ad libitum access to food. In agreement with the previous reports in the literature (e.g. Cartmell et al., 1999), LY354740 (10 mg/kg) now did reduce the hyperactivity following amphetamine (2.5 mg/kg) administration in these animals (Fig. 2B).

As expected, amphetamine significantly increased locomotor activity (Amph , , Amph x Time , ), an effect which was significantly reduced by LY354740 (Amph x LY354740 x Time , , Amph x LY354740 , ). Specifically, LY354740 significantly reduced Amph induced hyperactivity 1-60 mins post injection (Amph/LY354740 vs Amph/Veh , ) but not 61-120 mins post injection (, ). In contrast, LY354740 alone did not affect locomotor activity (Veh/LY354740 vs Veh/Veh, 1-60 mins , , 61-120 mins , ).



**Figure 2**. The effect of LY354740 and amphetamine on locomotor activity in **(A)** food restricted (experiment 3) and **(B)** ad libitum fed rats (experiment 4). Activity was measured by the number of infra-red beam breaks in locomotor activity arena. Time is presented in bins of 10 minutes relative to the time of injection, and the pre-injection 60 min period of habituation to the locomotor box is indicated by light grey highlight (left). Rats received injections consisting of either vehicle (Veh/Veh), vehicle and 10 mg/kg LY354740 (Veh/LY354740), vehicle and 2.5 mg/kg amphetamine (Amph/Veh), 2.5 mg/kg amphetamine and 10 mg/kg LY354740 (Amph/LY354740). Error bars represent +/- SEM. Statitsical significance of post-hoc simple effects (Tukey corrected), following significant interactions, were conducted on contrasts aggregating over 60 minute time bins (i.e. bins 1-6 and 7-12) to minimise family-wise error rate inflation with repeated testing: \* = *p* < .05, \*\* = *p* < .001.

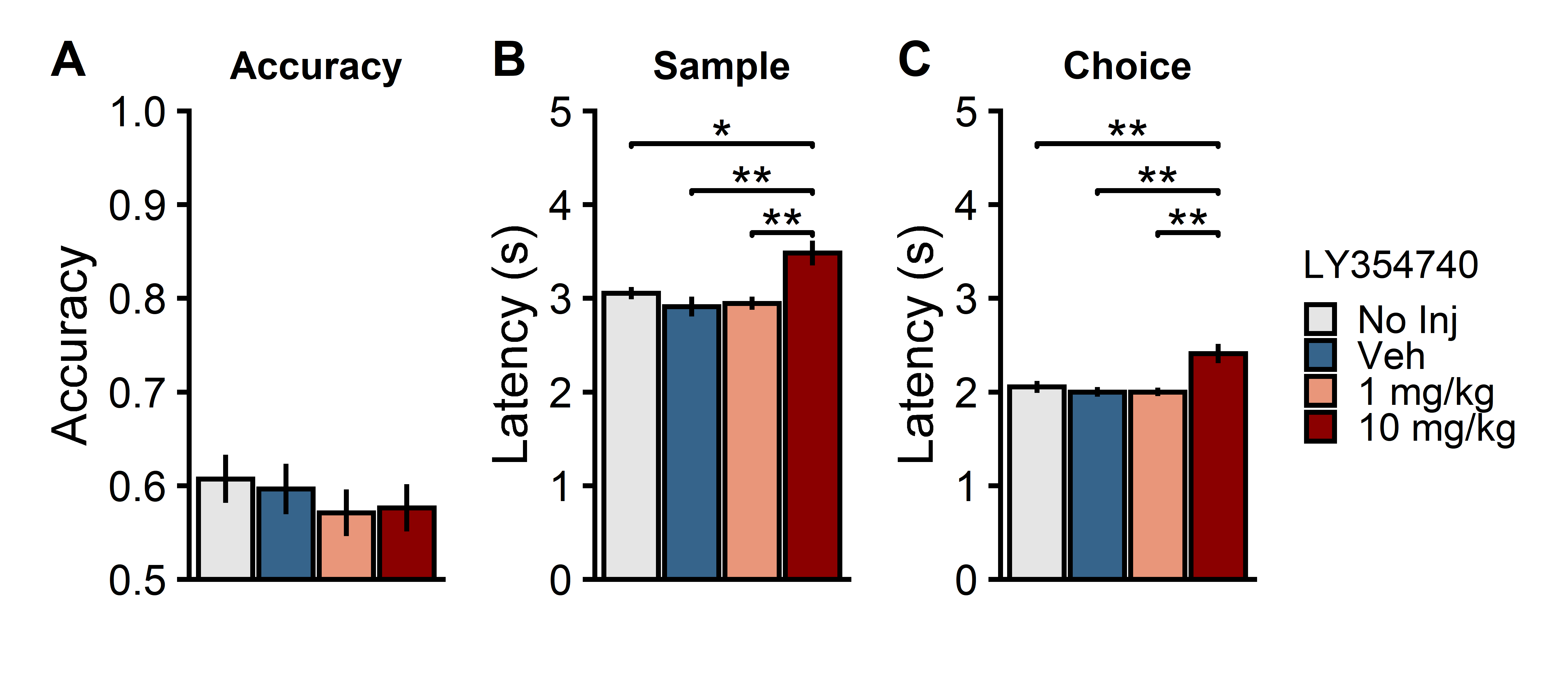
## Experiment 5: LY354740 increased phasic dopamine responses in the nucleus accumbens to reward

## Experiment 5: LY354740 did not affect phasic dopamine responses in the nucleus accumbens in anaesthetised rats

## Supplementary results

### Experiment 1 - 40s delay

Increasing the delay between sample and test to 40s resulted in lower accuracy levels (from approximately 80 to 60% in control conditions, Fig. 1A, Fig. S1A). While there was no significant disruption of accuracy by 10 mg/kg LY354740 (Fig. S1A; main effect of Drug, , ), response latencies were significantly increased during both the sample and choice periods (Fig. S1B; Sample period main effect of Drug , ; No Inj vs 10 mg/kg , , Veh vs 10 mg/kg , , 1 mg/kg vs 10 mg/kg , ; Choice period main effect of Drug , ; No Inj vs 10 mg/kg , , Veh vs 10 mg/kg , , 1 mg/kg vs 10 mg/kg , ).



**Figure S1**. The effect of LY354740 on spatial working memory in rewarded alternation T-maze assay on trials with a 40s delay. Trials with a 40s delay between sample and choice were randomly interleaved throughout Experiment 1. The effect of either no injection (No Inj), distilled water (Veh), 1 mg/kg LY354740, or 10 mg/kg LY354740 on accuracy and response latencies on these trials are presented here. **(A)** Performance accuracy measured as the proportion of trials with a correct side alternation from sample to choice. Latency (s) to enter the left or right arm of the T-maze during the sample **(B)** and choice **(C)** periods. Error bars represent +/- SEM. Statitsical significance of post-hoc simple effects (Tukey corrected) following a significant main effect of Drug: \* = *p* < .05, \*\* = *p* < .001.

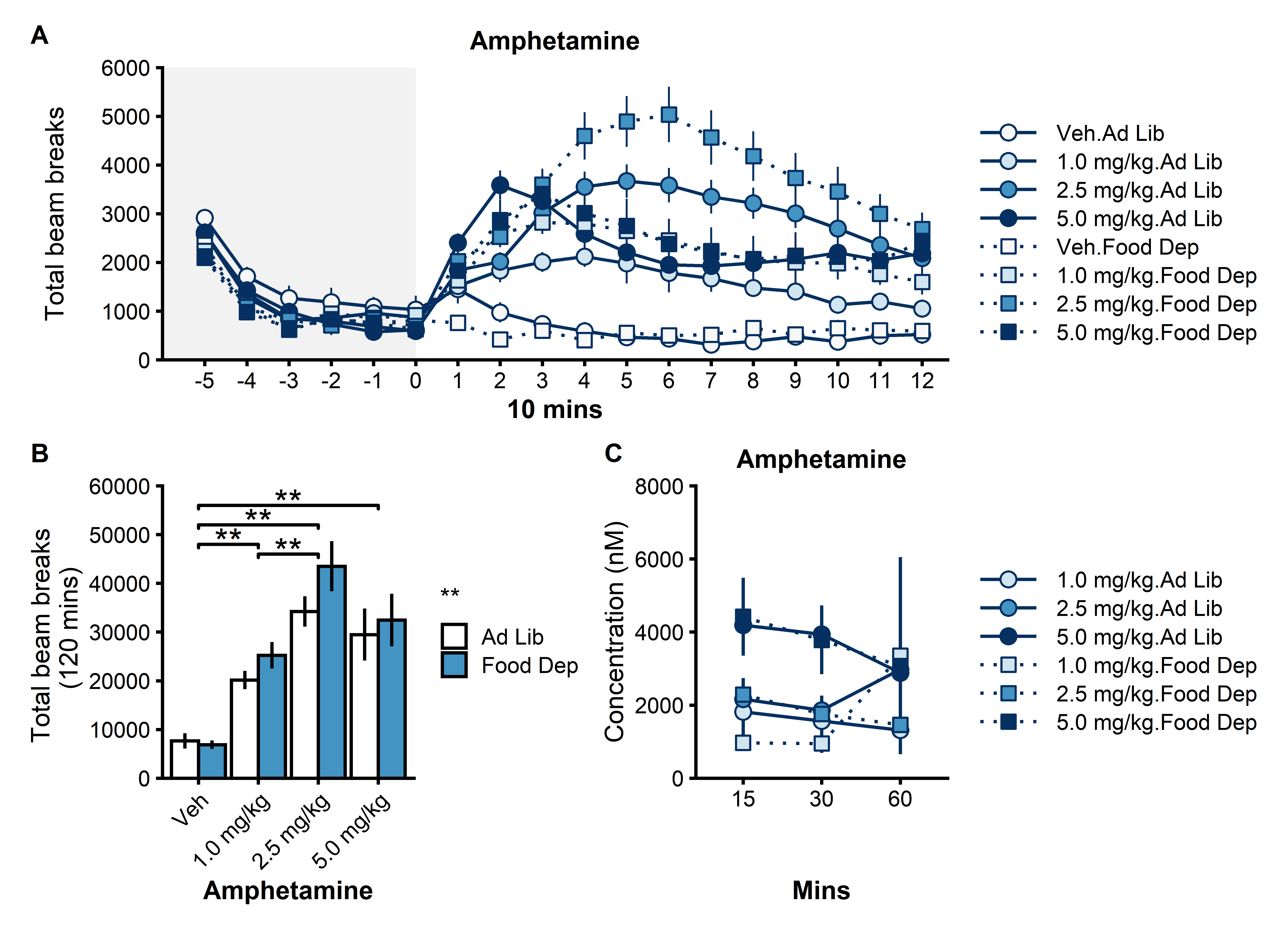
### Effect of feeding state on amphetamine hyper-locomotion and blood amphetamine levels

# Locomotor Activity Dose Amphetamine Feeding Manipulation

One potential explanation of the differential behavioural effects of LY354740 on amphetamine in food restricted and ad libitum fed rats is that food restriction changes the absorption, distribution, metabolism, or excretion profile of d-amphetamine. To test this possibility a new cohort of 90 rats were tested for the effect of feeding state on amphetamine hyper-locomotion (food restricted or ad libitum food access and i.p. injection of veh, 1.0 mg/kg, 2.5 mg/kg, or 5.0 mg/kg amphetamine; between subjects design). Prior to Amph administration (Fig. S2A, left), locomotor activity was significantly lower in food restricted than Ad libitum food access rats (Feeding , , Feeding x Time , ) confirming the effect of feeding state on arousal or attentional processes that drive exploratory lcomotor activity.

Following injections (Fig. S2A), both food restriction (Feeding , , Feeding x Time , ) and amphetamine (Amph , , Amph x Time , ) independently enhanced locomotor activity, however they did not significantly interact with each other (Amph x Feeding x Time , , Amph x Feeding , , ). Varying doses of amphetamine produced a dose response curve (Fig. S2B, total activity post injection) such that all doses of amphetamine increased activity relative to vehicle injections (0 vs 1.0 mg/kg , , 0 vs 2.5 mg/kg , , 0 vs 5.0 mg/kg , ), and 2.5 mg/kg produced the highest response (1.0 vs 2.5 mg/kg , , 2.5 vs 5.0 mg/kg , , 1.0 vs 5.0 mg/kg , ).

Next, blood was sampled at 15, 30, and 60 mins post injection in a cohort of 24 rats that were either food restrcited or had ad libitum food access and an i.p. injection of either 1.0 mg/kg, 2.5 mg/kg, or 5.0 mg/kg amphetamine (Fig. S2C). Blood levels of amphetamine increased with injection dose (Fig. S2C; main effect of Amph , , 1.0 vs 2.5 mg/kg , , 1.0 vs 5.0 mg/kg , , 2.5 vs 5.0 mg/kg , ; No main effect of Time , , or Amph x Time interaction , ). However, feeding state did not signficantly affect blood levels of amphetamine (Feeding , , Feeding x Time , ), or interact with amphetamine dose (Amph x Feeding , , Feeding x Amph x Time , ). This suggests that changes in amphetamine absorption, metabolism, or excretion are unlikely to account for the interaction between feeding state and hyper-locomotion or the differential effects of LY354740 in experiments 3 and 4 (Fig. 2).



**Figure S2**.**(A)** The effect of food restriction and ad libitum food access on amphetamine-induced locomotor activity in. Activity was measured by the number of infra-red beam breaks in locomotor activity arena. Time is presented in bins of 10 minutes relative to the time of injection, and the pre-injection 60 min period of habituation to the locomotor box is indicated by light grey highlight (left). Food restricted (Food Dep) and ad libitum fed rats (Ad Lib) received injections consisting of either vehicle (Veh), 1.0 mg/kg, 2.5 mg/kg, or 5 mg/kg amphetamine. **(B)** Total activity post-injection summarising data presented in **(A)**. Statitsical significance of post-hoc simple effects (Tukey corrected), following significant main effects: \* = *p* < .05, \*\* = *p* < .001. **(C)** Blood amphetamine levels (nM) sampled 15, 30, and 60 mins post injection of 1.0 mg/kg, 2.5 mg/kg, or 5 mg/kg amphetamine. Error bars represent +/- SEM.