2018

Liraglutide prevents metabolic side-effects and improves recognition and working memory during antipsychotic treatment in rats

Ilijana Babic
University of Wollongong, *Illawarra and Shoalhaven Local Health District*, ib171@uowmail.edu.au

Ashleigh Gorak
University of Wollongong, ag676@uowmail.edu.au

Martin Engel
University of Wollongong, mengel@uow.edu.au

Dominic Sellers
University of Wollongong, das752@uowmail.edu.au

Paul Else
University of Wollongong, pelse@uow.edu.au

See next page for additional authors

Publication Details

Babic, I., Gorak, A., Engel, M., Sellers, D., Else, P., Osborne, A. L., Pai, N., Huang, X., Nealon, J. & Weston-Green, K. (2018). Liraglutide prevents metabolic side-effects and improves recognition and working memory during antipsychotic treatment in rats. Journal of Psychopharmacology, 32 (5), 578-590.

Liraglutide prevents metabolic side-effects and improves recognition and working memory during antipsychotic treatment in rats

Abstract

BACKGROUND: Antipsychotic drugs (APDs), olanzapine and clozapine, do not effectively address the cognitive symptoms of schizophrenia and can cause serious metabolic side-effects. Liraglutide is a synthetic glucagon-like peptide-1 (GLP-1) receptor agonist with anti-obesity and neuroprotective properties. The aim of this study was to examine whether liraglutide prevents weight gain/hyperglycaemia side-effects and cognitive deficits when co-administered from the commencement of olanzapine and clozapine treatment. METHODS: Rats were administered olanzapine (2 mg/kg, three times daily (t.i.d.)), clozapine (12 mg/kg, t.i.d.), liraglutide (0.2 mg/kg, twice daily (b.i.d.)), olanzapine + liraglutide co-treatment, clozapine + liraglutide co-treatment or vehicle (Control) (n = 12/group, 6 weeks). Recognition and working memory were examined using Novel Object Recognition (NOR) and T-Maze tests. Body weight, food intake, adiposity, locomotor activity and glucose tolerance were examined. RESULTS: Liraglutide co-treatment prevented olanzapine- and clozapine-induced reductions in the NOR test discrimination ratio ($p < 0.001$). Olanzapine, but not clozapine, reduced correct entries in the T-Maze test ($p < 0.05$ versus Control) while liraglutide prevented this deficit. Liraglutide reduced olanzapine-induced weight gain and adiposity. Olanzapine significantly decreased voluntary locomotor activity and liraglutide co-treatment partially reversed this effect. Liraglutide improved clozapine-induced glucose intolerance. CONCLUSION: Liraglutide co-treatment improved aspects of cognition, prevented obesity side-effects of olanzapine, and the hyperglycaemia caused by clozapine, when administered from the start of APD treatment. The results demonstrate a potential treatment for individuals at a high risk of experiencing adverse effects of APDs

Publication Details

Babic, I., Gorak, A., Engel, M., Sellers, D., Else, P., Osborne, A. L., Pai, N., Huang, X., Nealon, J. & Weston-Green, K. (2018). Liraglutide prevents metabolic side-effects and improves recognition and working memory during antipsychotic treatment in rats. Journal of Psychopharmacology, 32 (5), 578-590.

Authors

Ilijana Babic, Ashleigh Gorak, Martin Engel, Dominic Sellers, Paul Else, Ashleigh L. Osborne, Nagesh B. Pai, Xu-Feng Huang, Jessica R. Nealon, and Katrina Weston-Green

Title: Liraglutide prevents metabolic side-effects and improves recognition and working memory during antipsychotic treatment in rats

Running head: Liraglutide prevents APD obesity and cognitive deficits

Authors: Ilijana Babic [a,b,c,d], Ashleigh Gorak [b,d], Martin Engel [a,b], Dominic Sellers [b,d], Paul Else [b,d], Ashleigh L. Osborne [a,b,d], Nagesh Pai [b,c,d], Xu-Feng Huang [a,b,d], Jessica Nealon [b,d] and *Katrina Weston-Green [a,b,d]

Affiliations: [a] Centre for Medical and Molecular Bioscience, Faculty of Science, Medicine and Health, University of Wollongong NSW Australia 2522, [b] Illawarra Health and Medical Research Institute, Wollongong, NSW Australia 2522, [c] Illawarra and Shoalhaven Local Health District, Wollongong, NSW Australia 2500, [d] School of Medicine, Faculty of Science, Medicine and Health, University of Wollongong NSW Australia 2522,

***Corresponding Author:** Dr Katrina Weston-Green
School of Medicine,
Faculty of Science, Medicine and Health
University of Wollongong, NSW Australia 2522
Email: kweston@uow.edu.au
Phone: + 61 2 4252 8506

Abbreviations: APD: antipsychotic drug, b.i.d: twice-daily, GLP-1: glucagon-like peptide-1, GLP-1R: glucagon-like peptide-1 receptor, SC: subcutaneous, T2DM: Type 2 Diabetes Mellitus, t.i.d: three times daily

Abstract:

Background: Antipsychotic drugs (APDs), olanzapine and clozapine, do not effectively address the cognitive symptoms of schizophrenia and can cause serious metabolic side-effects. Liraglutide is a synthetic glucagon-like peptide-1 (GLP-1) receptor agonist with anti-obesity and neuroprotective properties. **Aim:** To examine whether liraglutide prevents weight gain/hyperglycaemia side-effects and cognitive deficits when co-administered from the commencement of olanzapine and clozapine. **Methods**: Rats were administered olanzapine (2mg/kg, t.i.d.), clozapine (12mg/kg, t.i.d.), liraglutide (0.2mg/kg, b.i.d.), olanzapine+liraglutide co-treatment, clozapine+liraglutide co-treatment or vehicle (control) (n=12/group, 6 weeks). Recognition and working memory were examined using Novel Object Recognition (NOR) and T-maze tests. Body weight, food intake, adiposity, locomotor activity and glucose tolerance were examined. **Results:** Liraglutide co-treatment prevented olanzapine- and clozapine-induced reductions in the NOR test discrimination ratio ($p<0.001$). Olanzapine, but not clozapine, reduced correct entries in the T-maze test ($p<0.05$ vs control) while liraglutide prevented this deficit. Liraglutide reduced olanzapine-induced weight gain and adiposity. Olanzapine significantly decreased voluntary locomotor activity and liraglutide co-treatment partially reversed this effect. Liraglutide improved clozapine-induced glucose intolerance. **Conclusion**: Liraglutide co-treatment improved aspects of cognition, prevented obesity side-effects of olanzapine, and the hyperglycaemia caused by clozapine, when administered from the start of APD treatment. The results demonstrate a potential treatment for individuals at a high risk of experiencing adverse effects of APDs.

Key words: cognition, glucagon-like peptide-1, liraglutide, antipsychotic, obesity

1. Introduction

Cognitive impairment, including deficits in recognition and working memory, affects 80% of people with schizophrenia (2006; Lee and Park, 2005; Pelletier et al., 2005). Cognition is recognised as a core component of the disorder from which other symptom domains (positive and negative symptoms) arise (Kahn and Keefe, 2013) and can be a predictor of functional outcome (Lepage et al., 2014). Despite the importance and prevalence of cognitive deficits, antipsychotic drugs (APDs) are limited in their ability to treat this symptom domain. For example, the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) and other studies have reported minimal or no overall effect of olanzapine, clozapine, risperidone, ziprasidone, perphenazine or quetiapine fumarate on cognition (Keefe et al., 2007; Nielsen et al., 2015). In fact, several authors argue that APDs may worsen cognition, particularly typical 1st generation APDs (Woodward et al., 2007; Kasper and Resinger, 2003). Similar findings have been reported in rodent studies, with either no effect (Kamei et al., 2006), or impaired working and recognition memory following olanzapine and clozapine administration (Levin et al., 2005; Castro et al., 2007; Addy and Levin, 2002; Levin and Christopher, 2006; Ortega-Alvaro et al., 2006). On the contrary, olanzapine, clozapine and ziprasidone attenuate cognitive deficits following phencyclidine and MK-801 treatment in rodents (Abdul-Monim et al., 2006; Karasawa et al., 2008).

Atypical (2nd generation) APDs (particularly olanzapine and clozapine) can also cause weight gain and hyperglycaemia side-effects that can lead to obesity and Type 2 diabetes mellitus (T2DM), as well as poor medication compliance (Weston-Green et al., 2013; Das et al., 2012;

74 Leucht et al., 2009). Furthermore, T2DM and obesity can exacerbate cognitive impairment in
75 schizophrenia patients (Han et al., 2013; Lindenmayer et al., 2012). Overall, novel
76 therapeutic approaches for the treatment of cognitive impairment in schizophrenia and the
77 prevention of metabolic side-effects associated with current medications are required.
78
79 Synthetic analogues of the incretin hormone glucagon-like peptide-1 (GLP-1), such as
80 liraglutide and exenatide, can attenuate hyperglycaemia in T2DM and reduce diabetes-
81 associated weight gain (Garber et al., 2011; Robinson et al., 2013; de Wit et al., 2014).
82 Several studies suggest that liraglutide can improve metabolic parameters during APD
83 treatment. For example, a liraglutide increased glucose tolerance and reduced body weight
84 during a recent clinical trial of overweight pre-diabetic individuals schizophrenia spectrum
85 disorder treated with olanzapine or clozapine (Larsen et al., 2017). Liraglutide also induced
86 weight loss and restored glucose homeostasis in an earlier case study of a schizophrenia
87 patient treated with clozapine (Ishøy et al., 2014), and in rats with established obesity and
88 T2DM caused by chronic olanzapine administration (Lykkegaard et al., 2008). Therefore,
89 there is evidence to suggest that liraglutide can treat established metabolic side-effects of
90 olanzapine and clozapine; however, it is unclear whether liraglutide can prevent the onset of
91 weight gain and glucose imbalance when combined with APDs from the start of treatment. In
92 addition to its metabolic benefits, the GLP-1 signalling system in the brain plays an important
93 role in learning, memory and neuroprotection (see reviews by Holscher, 2013; Holst et al.,
94 2011). Endogenous GLP-1 is expressed in the nucleus of the solitary tract (NST) that project
95 throughout the brain (Hamilton and Holscher, 2009). Exogenous glucagon-like peptide-1
96 receptor (GLP-1R) agonists, liraglutide, lixisenatide and exendin-4, can exert central effects
97 by rapidly crossing the blood brain barrier (Hunter and Holscher, 2012; Kastin and
98 Akerstrom, 2003). GLP-1Rs are widely expressed in the brain, including regions associated

with cognitive function such as the hippocampus, amygdala, prefrontal cortex and nucleus accumbens (Katsurada and Yada, 2016; Hamilton and Holscher, 2009; Larsen and Holst, 2005; Merchenthaler et al., 1999). Interestingly, GLP-1-R knockout mice exhibit impaired recognition and spatial memory during the novel object recognition (NOR) and Morris water maze tests, as well as reduced long-term potentiation in the hippocampal CA1 region, compared to wild-type littermates (Abbas et al., 2009). Conversely, hippocampal GLP-1R overexpression improves memory and learning (During et al., 2003). Liraglutide prevents memory impairments, preserves synapses and synaptic plasticity, while exenatide improves short and long-term memory and spatial learning in mouse models of Alzheimer's disease (McClean et al., 2011; Bomba et al., 2013). However, whether liraglutide can improve cognition when administered from the start of APD treatment is unknown. The aim of this study was to investigate 1) the effect of liraglutide co-treatment on learning, working and recognition memory, and 2) the ability of liraglutide to prevent metabolic side-effects, in rats commencing olanzapine or clozapine treatment.

2. Methods

2.1 Ethics Statement

This study is reported in accordance with the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines (Kilkenny et al., 2010). The completed 'ARRIVE Guidelines Checklist' is included in the Supplementary Information (Supplementary Figure S1). All experimental procedures were approved by the Animal Ethics Committee, University of Wollongong, Australia (AE14/30), and complied with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (NHMRC, 2013). Every effort was made to minimize the number of animals and their suffering in this study.

2.2 Animals and treatment

Female Sprague-Dawley rats (200 – 220 g, Animal Resource Centre, Perth, WA, Australia) (n=72) were individually housed on corn cob bedding, with plastic tunnels and nesting material for environmental enrichment. The housing environment was set to 22 °C with a reverse light-dark cycle (photophase 1900-0700 h) so that behavioural experiments were conducted during the normal nocturnal active period of rats. *Ad libitum* access to water and standard laboratory chow (3.9 kcal/g; fat 10%, carbohydrates 74% and protein 16%) was provided throughout the study unless otherwise stated. An initial one-week environmental habituation period was followed by a training week where rats learnt to self-administer cookie dough pellets offered by researchers on a metal spatula, as previously described (Weston-Green et al., 2011). Rats were randomised into six treatment groups: 0.2 mg/kg liraglutide (Victoza, Novo Nordisk, Bagsværd, Denmark) (subcutaneous (SC) injection + oral cookie dough pellet without drug), 2 mg/kg olanzapine (Zyprexa, Eli Lilly, Indianapolis, IN, USA) (oral cookie dough pellet + sterile water SC), 12 mg/kg clozapine (Clozaril, Norvatis, Basel, Switzerland) (oral cookie dough pellet + sterile water SC), olanzapine+liraglutide co-treatment, clozapine+liraglutide co-treatment, or vehicle (oral cookie dough pellet without drug + sterile water (SC)) (n=12). Drug preparation was performed as detailed in Weston-Green et al (2011). Briefly, de-coated olanzapine and clozapine tablets were pulverized and the assigned doses were added to a dry cookie-dough mix. Immediately prior to administration, water was added to achieve a dry-dough consistency and rats were administered a 0.3 g cookie-dough pellet. Treatments were administered for six weeks (Figure 1A). Clinical administration routes were utilised, and clinical doses were converted to rat equivalents using body surface area calculations (Reagan-Shaw et al., 2008) and based on previous reports (Sturis et al., 2003; Weston-Green et al., 2011). APDs were administered three times daily (t.i.d) at 8-hourly intervals (0700, 1500 and 2300 h), while liraglutide was

149 administered twice daily (b.i.d) (0700 and 1500 h), based on the pharmacokinetic half-life of
150 these drugs in the rat (Aravagiri et al., 1999; Baldessarini et al., 1993; Sturis et al., 2003).
151 Cookie dough preparation and administration were conducted as we have previously
152 described (Weston-Green et al., 2011). Body weight, food and water intake were measured
153 weekly. Food intake was measured by weighing the amount of chow remaining in the hopper,
154 including any chow dispersed throughout the cage floor.
155
156 **2.3 Oral Glucose Tolerance Test (OGTT)**
157 An OGTT was performed during week 2 of treatment. Rats were fasted overnight then fasting
158 (baseline, time 0) blood glucose levels were measured using samples obtained from the
159 lateral tail vein. Measurements were obtained using a handheld glucometer (Accu-Chek
160 Performa Blood Glucose Meter, Sydney, Australia) in triplicate. Rats then received an oral
161 bolus of 2 g/kg glucose and blood sampling was repeated at 30 minute intervals until 180
162 mins after glucose administration. The mean blood glucose area under the curve (AUC)
163 OGTT blood glucose concentration was calculated using the trapezoidal method, as
164 previously described (Purves, 1992). Treatment groups were alternated throughout the testing
165 period.
166
167 **2.4 Behavioural Testing**
168 Behavioural testing was performed during weeks 3-5 of treatment to assess 24-hour voluntary
169 locomotor activity, memory and learning. Testing was conducted during the active dark phase
170 (0900-1400 hours) with minimal white light interference and a 24-hour rest period between
171 tests to minimise animal stress. Rats were tested from alternating treatment groups
172 throughout the testing period. Rat behaviour was recorded using standard commercial
173 cameras (Logitech Pty Ltd, NSW Australia) and recordings were de-identified. Equipment

was cleaned between trials to eliminate olfactory cues. To further minimise animal stress, behavioural tests were performed in order of invasiveness, as listed below.

2.4.1 Voluntary Locomotor Activity

Voluntary locomotor activity was measured using a rodent activity wheel with an integrated living chamber apparatus (Activity Wheel and Living Chamber, Model 80859, Lafayette Instrument Company, IN, USA). Rats were placed in clean cages equipped with freely spinning running wheels that were open to allow the rat a choice to either engage in voluntary locomotion or to reside in the living chamber (containing corncob bedding and free access to food and water) for 24-hours. Distance travelled (m) and velocity (m/sec) in the running wheel were determined using an infra-red sensor counter attached to a USB Computer Interface and quantified using Activity Wheel Monitor Software (Lafayette Instrument Company, IN, USA).

2.4.2 Novel Object Recognition (NOR) Test

The NOR test was employed to assess recognition memory, as demonstrated by the ability of a rat to distinguish between novel and familiar objects. The NOR test is driven by the rat's innate preference towards novelty (Cheng et al., 2014). Testing was conducted based on the protocol by Bevins and Besheer (2006) and our previous report (Osborne et al., 2017). Briefly, rats were provided with two objects (plastic building blocks) in their home cage for 24 hour familiarisation prior to the testing day. During the first (familiarisation) trial, each rat was placed in a dimly lit (even lighting of 25 lux) open arena (60 cm x 60 cm x 60 cm, black matte) with the two familiar objects positioned equidistant from the rat (Figure 1B). The two familiar objects were positioned in the upper half of the arena, while the rat was positioned in the lower half of the arena with head pointed towards the centre of the wall, away from the

objects (Figure 1B). The rat was allowed to explore the arena for 10 minutes then returned to its home cage for 1 hour. During the second (test) trial, one familiar object was replaced with a novel object (a toy figurine) in the arena (Figure 1B). The rat was returned to the arena and allowed to explore for 3 minutes. Object interaction time was recorded, defined as time spent nosing, sniffing, licking or touching the objects with forepaws. Inadvertent contact (rearing over object to explore other parts of the arena, bumping object in passing) were not included as object interaction, as described by Bevins and Besheer (2006). A discrimination ratio was calculated for each rat using T_N / T_{TOT} (T_N = novel object exploration time, T_{TOT} = total object exploration time (sec)), as previously described (Bevins and Besheer, 2006; Osborne et al., 2017). A discrimination ratio score of 1 indicated a preference for the novel object, whereas a score closer to 0 indicated a greater preference for the familiar object.

2.4.3 Rewarded T-maze Alternation Test

The T-maze alternation test was used to assess working memory based on the method described by Deacon and Rawlins (2006) and our previous report (Osborne et al., 2017). The maze consisted of a matte black 'T'-shaped arena (50 cm long x 10 cm wide, with 30 cm high walls) with removable dividers to allow access to the left and right goal arms of the upper part of the maze (Figure 1C). A 10cm central partition distal to the start arm was utilised to limit rat access to one goal arm of the T-maze at a time and improve alternation rate (Deacon and Rawlins, 2006). The maze was positioned at ground level and exposed to an even low light level of 20 lux. Rats were familiarised with a chocolate pellet reward stimulus in their home cages 24-hours prior to testing. Food was then restricted to 5 g / 100 g body weight of rat (approximately 40% of the normal intake) overnight prior to the habituation, training and testing days. During the habituation trial, the reward stimulus was placed in both open arms of the maze. A rat was placed in the start arm of the arena and allowed to freely explore the

maze for 10 minutes, then returned to its home cage. All rats were successfully habituated during this habituation trial as the reward stimulus was consumed from both arms of the T-maze (Deacon and Rawlins, 2006). Rats then underwent two consecutive days of training where they learnt to alternate entry into the left and right arms of the maze. Each training day involved 10 trials, including 5 'forced' and 5 'choice' runs. During the 'forced' run, one arm of the maze was closed using a removable divider and the reward stimulus was placed in the open arm (Figure 1C). The divider position was randomly alternated between the left and right arms of the maze for each forced run. During the 'choice' run, the divider was removed and the reward stimulus was placed in the newly opened arm (Figure 1C). Training was considered successful when control animals achieved greater than 80% correct entry (Deacon and Rawlins, 2006). The same alternation methods were utilised on the test day, with a total of 10 trials (5 forced and 5 choice runs) per rat. There was a 30 second delay between the forced and choice trials, and the total trial time was 3 minutes. A response was considered correct when the rat positioned its whole body in the correct arm (Deacon and Rawlins, 2006). Trials were excluded if the rat did not leave the start arm, jumped out of the maze or if the retention interval exceeded 45 seconds, as the duration of the interval between the forced and choice runs can impact cognitive performance (Freudenberg et al., 2013; Sharma et al., 2014; Deacon and Rawlins, 2006).

2.5 Post-Mortem Adiposity Measurement

After six weeks, animals were fasted overnight and euthanized via CO_2 asphyxiation. Subcutaneous inguinal, intra-abdominal perirenal and periovary white fat pads, and sub-scapula brown fat pads were individually dissected and weighed.

2.6 Statistical Analysis

Statistical analysis was performed using SPSS (version 21, SPSS, Chicago, IL, USA). Data were examined using Shapiro-Wilk tests for normality. Outliers ± 2 SD from the mean were removed, as previously published (Osborne et al., 2017). Two-way repeated ANOVAs (TREATMENT x TIME as repeated measures) were used to analyse cumulative weight gain, food and water intake, and glucose tolerance data. One-way ANOVAs were used to determine the effect of treatment on feeding efficiency, glucose AUC, voluntary locomotor activity (total distance travelled and velocity), NOR performance (discrimination ratio and total interaction time) and fat pad (inguinal, perirenal, periovary and sub-scapula) masses, followed by Tukey HSD and Dunnett-T tests for multiple comparisons (two-tailed). T-Maze data remained non-normally distributed despite log transformations, therefore, data were analysed using non-parametric Kruskall-Wallis followed by Mann-Whitney U tests for multiple comparisons. Comparisons included examination of changes in treatment groups compared to controls and differences between antipsychotic drug *vs* antipsychotic drug + liraglutide co-treatment groups. Correlations were identified using Pearson's correlation tests. Significance was accepted at $p<0.05$.

3. Results

3.1 Body weight, food and water intake

A two-way repeated ANOVA (TREATMENT x TIME) of cumulative body weight gain revealed a significant effect of treatment ($F_{5,61} = 13.866$, $p<0.001$) and time ($F_{5,57} = 42.056$, $p<0.001$), and a significant interaction between the two factors ($F_{25,213} = 2.045$, $p<0.01$). Compared to controls, olanzapine significantly increased body weight (weeks 1-6), while liraglutide co-treatment prevented this weight gain, with significantly decreased cumulative weight gain in the olanzapine + liraglutide group compared to the olanzapine treatment group ($p<0.01$ and $p<0.001$ throughout weeks 1-6) (Figure 2A). Liraglutide significantly decreased

body weight compared to the controls ($p<0.05$ and $p<0.01$ throughout weeks 1-4 and 6) (Figure 2A). As expected, clozapine did not alter body weight compared to the controls; however, clozapine + liraglutide co-treatment significantly decreased cumulative body weight during weeks 1 - 4 compared to the clozapine treatment group (Figure 2A). There was no significant effect of treatment on cumulative food intake, only an effect of time ($p<0.001$) (Figure 2B), and no alterations in cumulative water intake (Figure 2C).

3.2 Adiposity

There was a significant effect of treatment on subcutaneous inguinal ($F_{5,65} = 6.317$, $p<0.001$), intra-abdominal perirenal ($F_{5,64} = 14.229$, $p<0.001$), periovary ($F_{5,66} = 12.835$, $p<0.001$) and total white fat mass ($F_{5,65} = 13.854$, $p<0.001$) (Table 1). Compared to controls, liraglutide significantly reduced inguinal and perirenal (both $p<0.05$), periovary and total white fat mass (both $p<0.01$) (Table 1). Olanzapine significantly increased total white fat mass ($p<0.01$), including intra-abdominal peri-ovarian fat ($p<0.05$), while olanzapine + liraglutide co-treatment prevented this hyperadiposity, with significantly less inguinal ($p<0.05$), perirenal, periovarian ($p<0.001$) and therefore total fat mass ($p<0.01$) compared to the olanzapine group (Table 1). Clozapine did not alter fat mass; however, clozapine + liraglutide treatment reduced intra-abomdinal fat (perirenal and periovary) and total fat mass compared to controls (all $p<0.01$) and clozapine treatment alone ($p<0.001$, $p<0.05$ and $p<0.01$, respectively) (Table 1).

3.3 Oral Glucose Tolerance Test

There was a significant effect of treatment ($F_{5,55} = 15.766$, $p<0.001$) and time ($p<0.001$) on oral glucose tolerance test data, but no significant interaction between the two factors ($F_{30,202} = 1.392$, $p>0.05$) (Figure 3A). Clozapine-treated rats had significantly higher fasting blood

glucose levels (baseline, time 0) compared to controls ($p<0.05$), while the clozapine + liraglutide group had significantly lower, control-like fasting blood glucose levels ($p<0.001$) (Figure 3A). Hyperglycaemia was evident in the clozapine group throughout the test, a result that was echoed in the glucose area under the curve data ($p<0.05$ vs controls, Figure 3B), and failed to reach control levels even after 180 minutes ($p<0.05$ vs controls, Figure 3A). On the contrary, liraglutide co-treatment prevented the hyperglycaemia caused by clozapine ($p<0.001$ cloz+lira vs cloz, Figure 3B) and control levels were reached by the end of the test (Figure 3A). Liraglutide treatment significantly decreased the glucose area under the curve ($p<0.001$ *vs* controls) (Figure 3B); however, this result was caused by a decrease in blood glucose levels during 60, 90 and 120 minutes test intervals and glycaemic balance was restored by 150 and 180 minutes (Figure 3A). Olanzapine and olanzapine + liraglutide co-treatment groups did not differ to the controls throughout the test (Figure 3).

3.4 Voluntary Locomotor Activity

Olanzapine significantly reduced total distance travelled compared to the controls ($p<0.001$) (Figure 4). Liraglutide co-treatment with olanzapine significantly increased distance travelled compared to the olanzapine group (+75%, $p<0.05$); however, the distance in the co-treatment group was still significantly lower than the controls ($p<0.001$) (Figure 4A). A similar trend was observed in the velocity data, where olanzapine significantly reduced velocity compared to controls ($p<0.001$), and liraglutide co-treatment was able to improve velocity compared to the olanzapine group ($p<0.05$), but this improvement did not reach control levels (Figure 4B). Clozapine treatment did not significantly alter distance travelled or velocity compared to the controls; however, clozapine + liraglutide co-treatment significantly reduced these parameters compared to the controls ($p<0.01$) (Figure 4A and 4B). Interestingly, the liraglutide treatment group also exhibited reduced distance travelled and velocity compared to the controls

(p<0.05) (Figure 4). The distance travelled each hour over a 24-hour period is presented in Figures 4C and 4D).

3.5 Novel Object Recognition Test

There was a significant effect of treatment on the mean discrimination ratio ($F_{5,66}$ = 13.95, p<0.001) (Figure 5A), with a reduction in the olanzapine and clozapine groups (both p<0.001 *vs* controls), demonstrating reduced recognition memory in these rats. Interestingly, liraglutide co-treatment with olanzapine and clozapine prevented these deficits in recognition memory, restoring the discrimination ratio to control levels (both p>0.05 vs controls) and significantly improving the discrimination ratio compared to APD treatment alone (both p<0.001). Olanzapine and clozapine did not significantly impact total exploration time (p>0.05) (Figure 5B). In addition, there was a significant negative correlation between mean discrimination ratio and total white fat mass (g) (r=-0.43, p<0.001) (Figure 5C).

3.6 Rewarded T-maze Alternation Test

Olanzapine significantly reduced the mean percentage of correct entries in the rewarded T-maze alternation test compared to the controls (p<0.05), indicating that olanzapine-treated rats had impaired aspects of working memory. Performance by the olanzapine + liraglutide co-treatment group did not differ to the controls (p>0.05) (Figure 6). Although olanzapine + liraglutide co-treatment improved the mean percentage of correct entries by 17%, this did not reach significance compared to the olanzapine group (Figure 6). There were no significant treatment effects on mean percentage of correct entries in the remaining groups.

4. Discussion

348 The results of the present study show that liraglutide can prevent the obesity side-effects of
349 olanzapine and the chronic hyperglycaemia side-effects of clozapine when administered from
350 the start of treatment. A recent randomised clinical trial of schizophrenia patients treated with
351 either olanzapine (n=15) or clozapine (n=32), who were obese (average patient body mass
352 index of 33) and pre-diabetic found that liraglutide was able to treat already established
353 obesity in these patients (causing an average weight loss of -5.6kg) and improved glycaemic
354 balance by 23% (Larsen et al., 2017). On the other hand, administration of the long acting
355 GLP-1R agonist exenatide (once-weekly) had no effect on body weight in obese
356 schizophrenia patients (Ishøy et al., 2017b). The finding that a liraglutide intervention was
357 able to treat the weight gain and glucose intolerance side-effects of olanzapine was first
358 shown in rodents (Lykkegaard et al., 2008) and in a later case study (Ishøy et al., 2013);
359 however, no studies had examined whether liraglutide could prevent the onset of metabolic
360 side-effects when administered from the start of treatment. Therefore, the results of our study
361 show promise for improved treatment of patients initiating APD treatment who are at an
362 elevated risk of suffering metabolic side-effects.
363
364 Liraglutide induces weight loss, in-part, by reducing appetite and inhibitory effects on the
365 reward aspects of feeding behaviour (Holst, 2007; Pi-Sunyer et al., 2015); however, we did
366 not observe significant changes in food intake in treated rats suggesting that body weight
367 alterations were not due to hypophagia and reduced energy intake. Our previous studies
368 showed that olanzapine dramatically increased body weight but with modest increases in food
369 intake (Weston-Green et al., 2011; Weston-Green et al., 2012), demonstrating that appetite
370 did not fully account for the body weight increases. On the contrary, food intake has been
371 found to contribute to olanzapine-induced weight gain in a rat study using a different
372 treatment regime (Davoodi et al., 2009). We also examined voluntary locomotor activity as

373 reduced motivation towards exercise has been reported in people with schizophrenia
374 prescribed olanzapine and clozapine (Archie et al., 2003; Beebe et al., 2011) and GLP-1R
375 signalling influences mesolimbic reward and motivation pathways in the brain (Richard et al.,
376 2015). Liraglutide co-treatment increased voluntary locomotor activity by 75% compared to
377 the olanzapine group; however, control levels were not restored. Therefore, when considering
378 the basic energy balance equation (energy intake versus energy expenditure) these results
379 suggest that factors other than food intake and exercise are involved in the weight gain
380 caused by olanzapine in the present study, as well as the ability of liraglutide to prevent it.
381 However, pair feeding studies would be required to confirm. Weight changes were associated
382 with increased adiposity in the present study. Indeed, olanzapine promotes lipid accumulation
383 by upregulating the lipogenesis pathway (Albaugh et al., 2010), while liraglutide reduces
384 adipogenic and lipolytic markers (El Bekay et al., 2016). Liraglutide also reduced adiposity
385 below control levels when combined with clozapine, but food intake and locomotor activity
386 were unaltered in the clozapine groups. This is consistent with a previous report that
387 liraglutide lowers the respiratory quotient in obese people, indicating a switch towards
388 increased fat oxidation (van Can et al., 2014). Future investigation into treatment effects on
389 markers of lipid oxidation is warranted.
390
391 In the present study, cumulative body weight gain significantly increased with olanzapine,
392 but not clozapine treatment compared to the controls, coinciding with evidence over the past
393 14 years that rodents are resistant to clozapine-induced weight gain (Choi et al., 2007; Cooper
394 et al., 2008; Albaugh et al., 2006; Pouzet et al., 2003; Weston-Green et al., 2011).
395 Nonetheless, clozapine-induced hyperglycaemia side-effects can be modelled in rodents
396 (Tulipano et al., 2007); a result that was replicated in this study. Similar to our study, the
397 obesity and diabetes side-effects of olanzapine and clozapine can also manifest

simultaneously or independently in patients (Stahl et al., 2009). Clozapine caused fasting hyperglycaemia and impaired glucose clearance during the OGTT, which is consistent with clinical (Ishøy et al., 2013; Hägg et al., 1998) and animal studies (Boyda et al., 2010). Blood glucose levels in the clozapine group did not return to a homeostatic level by the end of the OGTT, indicative of poor insulin response to rising blood glucose levels and suggests the presence of a diabetic phenotype in these rats. Importantly, liraglutide co-treatment prevented these effects. Liraglutide alone induced lower weight gain than the controls, but rats in this group were normo-glycaemic, even following an overnight fast. This result is consistent with clinical studies reporting that liraglutide causes weight loss and has a low hypoglycemic risk (Feng et al., 2015; Lind et al., 2015). While clozapine increases glucagon secretion and stimulates hepatic glucose output (Smith et al., 2008), liraglutide activates GLP-1 receptors expressed on pancreatic α cells to inhibit the release of glucagon and suppress hepatic glucose output in a glucose-dependent manner (Steinert et al., 2016). In addition, GLP-1 delays gastric emptying (van Can et al., 2014), stimulates pancreatic beta cell proliferation and improves glucose sensitivity in these cells (Tamura et al., 2015). Furthermore, GLP-1 receptors are expressed in glucoreceptive regions of the brain that control blood glucose and food intake, including the caudal brainstem and hypothalamus (Alvarez et al., 2005), that may be altered by clozapine and liraglutide treatment.

This study is novel in its finding that liraglutide can prevent deficits in cognition that were associated with olanzapine and clozapine, when administered from the start of treatment. Olanzapine and clozapine impaired performance in the NOR test, while liraglutide co-treatment prevented these behavioural deficits. Olanzapine also caused deficits in T-Maze behaviour that were not evident in the olanzapine+liraglutide co-treatment group compared to controls. Liraglutide produced no changes in any of the behaviours examined when

423 administered alone, but was able to prevent APD-induced cognitive deficits with results
424 demonstrating control-like levels of performance in the co-treatment groups. The inability to
425 discriminate familiar compared to novel objects demonstrates a deficit in recognition memory
426 and learning (Bevins and Besheer, 2006), while impaired T-maze performance suggests
427 impaired working memory (Deacon and Rawlins, 2006), both of which are prominent
428 features of cognitive impairment in schizophrenia (McGuire et al., 2013). A recent clinical
429 study reported no cognitive benefits of long-acting exenatide (once-weekly) in patients with a
430 criteria of obesity, diagnosed schizophrenia spectrum disorder and a minimum of 3 months of
431 APD treatment (including typical 1st and atypical 2nd generation drugs as well as
432 polypharmacy) (Ishøy et al., 2017a). Several explanations for the confounding results may
433 include 1) a difference in dosage between the studies, 2) findings in healthy rodents may not
434 translate to humans with schizophrenia disorders, or 3) possible pharmacological differences
435 between long-acting exenatide and liraglutide. On the latter two points, the same group also
436 reported no effects of long-acting exenatide on body weight in a similar clinical study (Ishøy
437 et al., 2017b); however, liraglutide induced persistent weight loss and metabolic benefits in
438 an earlier case study of an obese patient with schizophrenia treated with clozapine (Ishøy et
439 al., 2013), a result translated from rats (Lykkegaard et al., 2008). Another explanation may be
440 that our prevention study employed liraglutide co-treatment from the start of APD treatment.
441 Studies show that liraglutide can cross the blood brain barrier, promote neuronal stem cell
442 proliferation and neurogenesis, and provides neuroprotective effects (Hunter and Holscher,
443 2012), including preserved synaptic number and function, and reduced neuroinflammation
444 (McClean and Hölscher, 2014; McClean et al., 2011). In other studies, liraglutide normalised
445 object recognition in a mouse model of Alzheimer's disease after 8 months of treatment
446 (McClean et al., 2015), exenatide and exendin-4 improved memory and spatial learning in the
447 Morris Water Maze in models of Alzheimer's disease and cognitive deficits induced by

448 impaired central insulin signalling (Bomba et al., 2013; Chen et al., 2012), and recognition
449 memory was improved by liraglutide (0.2mg/kg, b.i.d.) in mice following a 20-week high fat
450 diet (Porter et al., 2010). On the other hand, APDs olanzapine and risperidone exacerbated
451 deficits in spatial working memory and attention during the Oculomotor Delayed Response
452 task (Reilly et al., 2007) and decreased dorsolateral prefrontal cortex activation during motor
453 learning tasks (Keedy et al., 2015) in previously APD-naïve schizophrenia patients after 4-6
454 weeks of treatment. In rodents, olanzapine impaired working memory during Y-Maze and
455 Eight-Arm Radial Maze tests (Castro et al., 2007; Levin et al., 2005; Ortega-Alvaro et al.,
456 2006), and reduced memory acquisition and retrieval in mice during the modified Elevated
457 Plus Maze task (Mutlu et al., 2011). Clinically, APDs can cause varying degrees of sedation
458 (Leucht et al., 2009) and it is possible that sedation impacted the cognitive data. However, in
459 the present study neither APD affected overall exploration time in the NOR test and
460 clozapine treated animals displayed impaired NOR test performance without exhibiting
461 hypolocomotor activity. In addition, treatment effects on reward and motivation may have
462 also played a role in cognitive test performance in the present study.
463
464 The underlying mechanisms by which GLP-1 analogues exert beneficial effects on cognition
465 in the brain remain unclear. Interestingly, studies have shown that olanzapine decreases
466 hippocampal volume (Barr et al., 2013) and lowers hippocampal connectivity (i.e. reduces
467 inhibitory terminal immunodensity) in rats (Ramos-Miguel et al., 2015). On the other hand,
468 neuronal GLP-1 has growth characteristics similar to insulin-like growth factor (see review
469 by Bassil et al., 2014), which may enable it to stimulate neurogenesis and hippocampal
470 plasticity to improve cognition. GLP-1 is also a neurotransmitter (Larsen and Holst, 2005),
471 with GLP-1Rs expressed in regions of the brain that are both implicated in cognition and
472 house major neurotransmitter pathways (dopamine, acetylcholine and serotonin) (i.e. the

frontal and temporal cortices, nucleus basalis Meynert, hippocampus, nucleus accumbens, amygdala, ventral tegmental area, substantia nigra, raphe nuclei and locus ceruleus) (Larsen and Holst, 2005; Merchenthaler et al., 1999). This anatomical arrangement suggests that the GLP-1 system interacts with other neurochemical systems in the brain. GLP-1 neurons of the NTS project to the nucleus accumbens and amygdala via the ventral tegmental area (i.e. the dopaminergic mesolimbic pathway) to alter the reward aspects of food intake (Alhadeff et al., 2012). In addition, central injection of exendin-4 increases dopamine turnover (upregulates levels of dopamine metabolites 3,4-dihydroxyphenylacetic acid and homovanillic acid) in the amygdala and this effect is abolished by D2 receptor antagonism (Anderberg et al., 2014), demonstrating an interaction between GLP-1 and D2 receptor signalling in a region of the brain implicated in reward, emotional processing and learning and memory. Olanzapine and clozapine are potent D2 receptor antagonists (McCormick et al., 2010), therefore, a GLP-1/D2 receptor interaction may be a potential mechanism by which liraglutide exerts its beneficial effects on cognition. The anti-cholinergic properties of olanzapine and clozapine may also contribute to cognitive dysfunction (McGurk et al., 2004), while exendin-4 upregulates choline acetyltransferase *in-vitro* and restores cholinergic neuron activity in the basal forebrain (involved in episodic memory retrieval in humans (Fujii et al., 2002)), in a rodent neurodegeneration model (Perry et al., 2002). Together with the behavioral findings of the present study, evidence suggests that liraglutide may restore control-like levels to neurotransmitter imbalances caused by APDs to improve cognition. However, liraglutide alone did not increase cognitive performance above control levels. Therefore, a second possibility is that the cognitive benefits of liraglutide were related to metabolic improvement. We identified a correlation between NOR test performance and adiposity and have previously shown that schizophrenia patients with comorbid diabetes had worsened cognitive performance than patients without diabetes (Han et al., 2013); a result echoed in

schizophrenia patients with metabolic syndrome (Lindenmayer et al., 2012). Therefore, further investigation into key neurotransmitter systems and the role of metabolic dysfunction in the cognitive benefits of liralgutide treatment observed in the present study are required.

In conclusion, liraglutide co-treatment from the start of APD administration prevented metabolic side-effects and cognitive deficits in rats. A limitation of this study is the use of healthy rodents and future studies may benefit from examining the efficacy of liraglutide in a schizophrenia pathology model prior to translation into humans. Overall, the results of this study provide evidence supporting the use of liraglutide as an adjunct therapy to improve the treatment of people with schizophrenia. This approach may particularly benefit patients already at a high risk of experiencing metabolic side-effects, including those commencing APD treatment with pre-existing T2DM and/or obesity, patients re-commencing APD treatment following non-compliance due to metabolic side-effects, or patients switching APDs in the presence of existing metabolic side-effects.

5. Acknowledgements

We thank Cree De Clouett, Anita Louise Obeid, Luisa Lufe, Paul Le Mesurier, Tereza Pejovska, Michael Green and Jeremy Shalala for their technical assistance in this project. Thank you to Tim Karl for critical input into the editing of this manuscript. We also gratefully acknowledge Eva Urban and the generosity of the staff at Suncorp Group Australia, as well as the Meyer family and the Schizophrenia Fellowship of NSW for their support.

6. Funding

This work was supported by Suncorp Group Limited Brighter Futures Community Giving (grant to KWG), the Peter Meyer Fund - Schizophrenia Fellowship of NSW (to KWG) and the Faculty of Science, Medicine and Health at the University of Wollongong (to KWG). The funding agencies had no input into the study design, outcomes or decision to publish the manuscript.

7. Conflict of Interests

The authors declare that they have no conflicts of interest.

8. References

Abbas T, Faivre E and Holscher C. (2009) Impairment of synaptic plasticity and memory formation in GLP-1 receptor KO mice: Interaction between type 2 diabetes and Alzheimer's disease. *Behav Brain Res* 205(1): 265-271.

Abdul-Monim Z, Reynolds GP and Neill JC. (2006) The effect of atypical and classical antipsychotics on sub-chronic PCP-induced cognitive deficits in a reversal-learning paradigm. *Behav Brain Res* 169(2): 263-273.

Addy N and Levin ED. (2002) Nicotine interactions with haloperidol, clozapine and risperidone and working memory function in rats. *Neuropsychopharmacology* 27(4): 534-541.

Albaugh VL, Henry CR, Bello NT, et al. (2006) Hormonal and metabolic effects of olanzapine and clozapine related to body weight in rodents. *Obesity (Silver Spring)* 14(1): 36-51.

Albaugh VL, Judson JG, She P, et al. (2010) Olanzapine promotes fat accumulation in male rats by decreasing physical activity, repartitioning energy and increasing adipose tissue lipogenesis while impairing lipolysis. *Molecular Psychiatry* 16(5): 569-581.

Alhadeff AL, Rupprecht LE and Hayes MR. (2012) GLP-1 Neurons in the Nucleus of the Solitary Tract Project Directly to the Ventral Tegmental Area and Nucleus Accumbens to Control for Food Intake. *Endocrinology* 153(2): 647-658.

Alvarez E, Martínez MD, Roncero I, et al. (2005) The expression of GLP-1 receptor mRNA and protein allows the effect of GLP-1 on glucose metabolism in the human hypothalamus and brainstem. *Journal of Neurochemistry* 92(4): 798-806.

Anderberg RH, Anefors C, Bergquist F, et al. (2014) Dopamine signaling in the amygdala, increased by food ingestion and GLP-1, regulates feeding behavior. *Physiology & Behavior* 136: 135-144.

Aravagiri M, Teper Y and Marder SR. (1999) Pharmacokinetics and tissue distribution of olanzapine in rats. *Biopharm Drug Dispos* 20(8): 369-377.

Archie S, Wilson J, Osborne S, et al. (2003) Pilot study: access to fitness facility and exercise levels in olanzapine-treated patients. *Canadian Journal of Psychiatry* 48(9): 628-632.

Baldessarini RJ, Centorrino F, Flood JG, et al. (1993) Tissue concentrations of clozapine and its metabolites in the rat. *Neuropsychopharmacology* 9(2): 117-124.

Barr AM, Wu CH, Wong C, et al. (2013) Effects of chronic exercise and treatment with the antipsychotic drug olanzapine on hippocampal volume in adult female rats. *Neuroscience* 255: 147-157.

Bassil F, Fernagut PO, Bezard E, et al. (2014) Insulin, IGF-1 and GLP-1 signaling in neurodegenerative disorders: targets for disease modification? *Prog Neurobiol* 118: 1-18.

Beebe LH, Smith K, Burk R, et al. (2011) Effect of a Motivational Intervention on Exercise Behavior in Persons with Schizophrenia Spectrum Disorders. *Community Mental Health Journal* 47(6): 628-636.

Bevins RA and Besheer J. (2006) Object recognition in rats and mice: a one-trial non-matching-to-sample learning task to study 'recognition memory'. *Nature Protocols* 1(3): 1306-1311.

Bomba M, Ciavardelli D, Silvestri E, et al. (2013) Exenatide promotes cognitive enhancement and positive brain metabolic changes in PS1-KI mice but has no effects in 3xTg-AD animals. *Cell Death & Disease* 4: e612.

Boyda HN, Tse L, Procyshyn RM, et al. (2010) A parametric study of the acute effects of antipsychotic drugs on glucose sensitivity in an animal model. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 34(6): 945-954.

Castro CC, Dos Reis-Lunardelli EA, Schmidt WJ, et al. (2007) Clozapine and olanzapine but not risperidone impair the pre-frontal striatal system in relation to egocentric spatial orientation in a Y-maze. *Curr Neurovasc Res* 4(4): 235-239.

Chen S, Liu AR, An FM, et al. (2012) Amelioration of neurodegenerative changes in cellular and rat models of diabetes-related Alzheimer's disease by exendin-4. *Age (Dordr)* 34(5): 1211-1224.

Cheng D, Low JK, Logge W, et al. (2014) Chronic cannabidiol treatment improves social and object recognition in double transgenic APPswe/PS1ΔE9 mice. *Psychopharmacology* 231(15): 3009-3017.

Choi S, DiSilvio B, Unangst J, et al. (2007) Effect of chronic infusion of olanzapine and clozapine on food intake and body weight gain in male and female rats. *Life Sciences* 81(12): 1024-1030.

Cooper GD, Harrold JA, Halford JC, et al. (2008) Chronic clozapine treatment in female rats does not induce weight gain or metabolic abnormalities but enhances adiposity: implications for animal models of antipsychotic-induced weight gain. *Prog Neuropsychopharmacol Biol Psychiatry* 32(2): 428-436.

Das C, Mendez G, Jagasia S, et al. (2012) Second-generation antipsychotic use in schizophrenia and associated weight gain: a critical review and meta-analysis of behavioral and pharmacologic treatments. *Ann Clin Psychiatry* 24(3): 225-239.

Davoodi N, Kalinichev M, Korneev S, et al. (2009) Hyperphagia and increased meal size are responsible for weight gain in rats treated sub-chronically with olanzapine. *Psychopharmacology* 203(4): 693-702.

de Wit HM, Vervoort GM, Jansen HJ, et al. (2014) Liraglutide reverses pronounced insulin-associated weight gain, improves glycaemic control and decreases insulin dose in patients with type 2 diabetes: a 26 week, randomised clinical trial (ELEGANT). *Diabetologia* 57(9): 1812-1819.

Deacon RMJ and Rawlins JNP. (2006) T-maze alternation in the rodent. *Nat. Protocols* 1(1): 7-12.

During MJ, Cao L, Zuzga DS, et al. (2003) Glucagon-like peptide-1 receptor is involved in learning and neuroprotection. *Nat Med* 9(9): 1173-1179.

El Bekay R, Coín-Aragüez L, Fernández-García D, et al. (2016) Effects of glucagon-like peptide-1 on the differentiation and metabolism of human adipocytes. *British Journal of Pharmacology* 173(11): 1820-1834.

Feng P, Yu D-m, Chen L-m, et al. (2015) Liraglutide reduces the body weight and waist circumference in Chinese overweight and obese type 2 diabetic patients. *Acta Pharmacologica Sinica* 36(2): 200-208.

Freudenberg F, Marx V, Seeburg PH, et al. (2013) Circuit mechanisms of GluA1-dependent spatial working memory. *Hippocampus* 23(12): 1359-1366.

Fujii T, Okuda J, Tsukiura T, et al. (2002) The role of the basal forebrain in episodic memory retrieval: a positron emission tomography study. *Neuroimage* 15(3): 501-508.

Garber A, Henry RR, Ratner R, et al. (2011) Liraglutide, a once-daily human glucagon-like peptide 1 analogue, provides sustained improvements in glycaemic control and weight for 2 years as monotherapy compared with glimepiride in patients with type 2 diabetes. *Diabetes Obes Metab* 13(4): 348-356.

Hägg S, Joelsson L, Mjörndal T, et al. (1998) Prevalence of diabetes and impaired glucose tolerance in patients treated with clozapine compared with patients treated with conventional depot neuroleptic medications. *Journal of Clinical Psychiatry*.

Hamilton A and Holscher C. (2009) Receptors for the incretin glucagon-like peptide-1 are expressed on neurons in the central nervous system. *Neuroreport* 20(13): 1161-1166.

Han M, Huang X-F, Chen DC, et al. (2013) Diabetes and Cognitive Deficits in Chronic Schizophrenia: A Case-Control Study. *PLoS ONE* 8(6): e66299.
Holscher C. (2013) Central effects of GLP-1: new opportunities for treatments of neurodegenerative diseases. *Journal of Endocrinology*.
Holst JJ. (2007) The physiology of glucagon-like peptide 1. *Physiological Reviews* 87(4): 1409-1439.
Holst JJ, Burcelin R and Nathanson E. (2011) Neuroprotective properties of GLP-1: theoretical and practical applications. *Current Medical Research and Opinion* 27(3): 547-558.
Hunter K and Holscher C. (2012) Drugs developed to treat diabetes, liraglutide and lixisenatide, cross the blood brain barrier and enhance neurogenesis. *BMC Neurosci* 13: 33.
Ishøy PL, Fagerlund B, Broberg BV, et al. (2017a) No cognitive-enhancing effect of GLP-1 receptor agonism in antipsychotic-treated, obese patients with schizophrenia. *Acta Psychiatrica Scandinavica* 136(1): 52-62.
Ishøy PL, Knop FK, Broberg BV, et al. (2014) Treatment of antipsychotic-associated obesity with a GLP-1 receptor agonist—protocol for an investigator-initiated prospective, randomised, placebo-controlled, double-blinded intervention study: the TAO study protocol. *BMJ Open* 4(1).
Ishøy PL, Knop FK, Broberg BV, et al. (2017b) Effect of GLP-1 receptor agonist treatment on body weight in obese antipsychotic-treated patients with schizophrenia: a randomized, placebo-controlled trial. *Diabetes, Obesity and Metabolism* 19(2): 162-171.
Ishøy PL, Knop FK, Vilsbøll T, et al. (2013) Sustained Weight Loss After Treatment With a Glucagon-Like Peptide-1 Receptor Agonist in an Obese Patient With Schizophrenia and Type 2 Diabetes *The American Journal of Psychiatry* 170(6): 681-682.
Kahn RS and Keefe RS. (2013) Schizophrenia is a cognitive illness: time for a change in focus. *JAMA Psychiatry* 70(10): 1107-1112.
Kamei H, Nagai T, Nakano H, et al. (2006) Repeated methamphetamine treatment impairs recognition memory through a failure of novelty-induced ERK1/2 activation in the prefrontal cortex of mice. *Biol Psychiatry* 59(1): 75-84.
Karasawa J, Hashimoto K and Chaki S. (2008) D-Serine and a glycine transporter inhibitor improve MK-801-induced cognitive deficits in a novel object recognition test in rats. *Behav Brain Res* 186(1): 78-83.
Kasper S and Resinger E. (2003) Cognitive effects and antipsychotic treatment. *Psychoneuroendocrinology* 28 Suppl 1: 27-38.
Kastin AJ and Akerstrom V. (2003) Entry of exendin-4 into brain is rapid but may be limited at high doses. *Int J Obes Relat Metab Disord* 27(3): 313-318.
Katsurada K and Yada T. (2016) Neural effects of gut- and brain-derived glucagon-like peptide-1 and its receptor agonist. *Journal of Diabetes Investigation* 7(Suppl Suppl 1): 64-69.
Keedy SK, Reilly JL, Bishop JR, et al. (2015) Impact of Antipsychotic Treatment on Attention and Motor Learning Systems in First-Episode Schizophrenia. *Schizophrenia Bulletin* 41(2): 355-365.
Keefe RE, Bilder RM, Davis SM, et al. (2007) Neurocognitive effects of antipsychotic medications in patients with chronic schizophrenia in the catie trial. *Arch Gen Psychiatry* 64(6): 633-647.
Kilkenny C, Browne WJ, Cuthill IC, et al. (2010) Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. *PLoS Biology* 8(6): e1000412.

Larsen JR, Vedtofte L, Jakobsen ML, et al. (2017) Effect of liraglutide treatment on prediabetes and overweight or obesity in clozapine- or olanzapine-treated patients with schizophrenia spectrum disorder: A randomized clinical trial. *JAMA Psychiatry* 74(7): 719-728.

Larsen PJ and Holst JJ. (2005) Glucagon-related peptide 1 (GLP-1): hormone and neurotransmitter. *Regulatory Peptides* 128(2): 97-107.

Lee J and Park S. (2005) Working memory impairments in schizophrenia: a meta-analysis. *Journal of Abnormal Psychology* 114(4): 599-611.

Lepage M, Bodnar M and Bowie CR. (2014) Neurocognition: Clinical and Functional Outcomes in Schizophrenia. *Canadian Journal of Psychiatry. Revue Canadienne de Psychiatrie* 59(1): 5-12.

Leucht S, Corves C, Arbter D, et al. (2009) Second-generation versus first-generation antipsychotic drugs for schizophrenia: a meta-analysis. *The Lancet* 373(9657): 31-41.

Levin ED and Christopher NC. (2006) Effects of clozapine on memory function in the rat neonatal hippocampal lesion model of schizophrenia. *Prog Neuropsychopharmacol Biol Psychiatry* 30(2): 223-229.

Levin ED, Petro A and Beatty A. (2005) Olanzapine interactions with nicotine and mecamylamine in rats: effects on memory function. *Neurotoxicol Teratol* 27(3): 459-464.

Lind M, Hirsch IB, Tuomilehto J, et al. (2015) Liraglutide in people treated for type 2 diabetes with multiple daily insulin injections: randomised clinical trial (MDI Liraglutide trial). *BMJ : British Medical Journal* 351.

Lindenmayer JP, Khan A, Kaushik S, et al. (2012) Relationship between metabolic syndrome and cognition in patients with schizophrenia. *Schizophrenia Research* 142(1-3): 171-176.

Lykkegaard K, Larsen PJ, Vrang N, et al. (2008) The once-daily human GLP-1 analog, liraglutide, reduces olanzapine-induced weight gain and glucose intolerance. *Schizophrenia Research* 103(1-3): 94-103.

McClean PL and Hölscher C. (2014) Liraglutide can reverse memory impairment, synaptic loss and reduce plaque load in aged APP/PS1 mice, a model of Alzheimer's disease. *Neuropharmacology* 76, Part A(0): 57-67.

McClean PL, Jalewa J and Holscher C. (2015) Prophylactic liraglutide treatment prevents amyloid plaque deposition, chronic inflammation and memory impairment in APP/PS1 mice. *Behav Brain Res* 293: 96-106.

McClean PL, Parthsarathy V, Faivre E, et al. (2011) The diabetes drug liraglutide prevents degenerative processes in a mouse model of Alzheimer's disease. *Journal of Neuroscience* 31(17): 6587-6594.

McCormick PN, Kapur S, Graff-Guerrero A, et al. (2010) The Antipsychotics Olanzapine, Risperidone, Clozapine, and Haloperidol Are D2-Selective Ex Vivo but Not In Vitro. *Neuropsychopharmacology* 35(8): 1826-1835.

McGuire KA, Blahnik MM and Sponheim SR. (2013) Discrimination within Recognition Memory in Schizophrenia. *Behavioral Sciences* 3(2): 273-297.

McGurk SR, Lee MA, Jayathilake K, et al. (2004) Cognitive effects of olanzapine treatment in schizophrenia. *MedGenMed* 6(2): 27.

Merchenthaler I, Lane M and Shughrue P. (1999) Distribution of pre-pro-glucagon and glucagon-like peptide-1 receptor messenger RNAs in the rat central nervous system. *The Journal of Comparative Neurology* 403(2): 261-280.

Mutlu O, Ulak G, Celikyurt IK, et al. (2011) Effects of olanzapine and clozapine on memory acquisition, consolidation and retrieval in mice using the elevated plus maze test. *Neurosci Lett* 501(3): 143-147.

NHMRC. (2013) Australian code for the care and use of animals for scientific purposes. . 8th Edition ed. Canberra Australian Government.
Nielsen RE, Levander S, Kjaersdam Telleus G, et al. (2015) Second-generation antipsychotic effect on cognition in patients with schizophrenia--a meta-analysis of randomized clinical trials. *Acta Psychiatr Scand* 131(3): 185-196.
Ortega-Alvaro A, Gibert-Rahola J and Micó JA. (2006) Influence of chronic treatment with olanzapine, clozapine and scopolamine on performance of a learned 8-arm radial maze task in rats. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 30(1): 104-111.
Osborne AL, Solowij N, Babic I, et al. (2017) Improved Social Interaction, Recognition and Working Memory with Cannabidiol Treatment in a Prenatal Infection (poly I:C) Rat Model. *Neuropsychopharmacology* 42(7): 1447-1457.
Pelletier M, Achim AM, Montoya A, et al. (2005) Cognitive and clinical moderators of recognition memory in schizophrenia: a meta-analysis. *Schizophrenia Research* 74(2–3): 233-252.
Perry T, Haughey NJ, Mattson MP, et al. (2002) Protection and Reversal of Excitotoxic Neuronal Damage by Glucagon-Like Peptide-1 and Exendin-4. *Journal of Pharmacology and Experimental Therapeutics* 302(3): 881-888.
Pi-Sunyer X, Astrup A, Fujioka K, et al. (2015) A Randomized, Controlled Trial of 3.0 mg of Liraglutide in Weight Management. *New England Journal of Medicine* 373(1): 11-22.
Porter DW, Kerr BD, Flatt PR, et al. (2010) Four weeks administration of Liraglutide improves memory and learning as well as glycaemic control in mice with high fat dietary-induced obesity and insulin resistance. *Diabetes Obes Metab* 12(10): 891-899.
Pouzet B, Mow T, Kreilgård M, et al. (2003) Chronic treatment with antipsychotics in rats as a model for antipsychotic-induced weight gain in human. *Pharmacology, Biochemistry and Behavior* 75(1): 133-140.
Purves RD. (1992) Optimum numerical integration methods for estimation of area-under-the-curve (AUC) and area-under-the-moment-curve (AUMC). *J Pharmacokinet Biopharm* 20(3): 211-226.
Ramos-Miguel A, Honer WG, Boyda HN, et al. (2015) Exercise prevents downregulation of hippocampal presynaptic proteins following olanzapine-elicited metabolic dysregulation in rats: Distinct roles of inhibitory and excitatory terminals. *Neuroscience* 301: 298-311.
Reagan-Shaw S, Nihal M and Ahmad N. (2008) Dose translation from animal to human studies revisited. *The FASEB Journal* 22: 659-661.
Reichenberg A, Weiser M, Caspi A, et al. (2006) Premorbid intellectual functioning and risk of schizophrenia and spectrum disorders. *J Clin Exp Neuropsychol* 28(2): 193-207.
Reilly JL, Harris MSH, Khine TT, et al. (2007) Antipsychotic Drugs Exacerbate Impairment on a Working Memory Task in First-Episode Schizophrenia. *Biol Psychiatry* 62(7): 818-821.
Richard JE, Anderberg RH, Göteson A, et al. (2015) Activation of the GLP-1 Receptors in the Nucleus of the Solitary Tract Reduces Food Reward Behavior and Targets the Mesolimbic System. *PLoS ONE* 10(3): e0119034.
Robinson LE, Holt TA, Rees K, et al. (2013) Effects of exenatide and liraglutide on heart rate, blood pressure and body weight: systematic review and meta-analysis. *BMJ Open* 3(1).
Sharma A, Shetty M, Parida A, et al. (2014) Effect of ethanolic extract of Acacia auriculiformis leaves on learning and memory in rats. *Pharmacognosy Research* 6(3): 246-250.

777 Smith G, Chaussade C, Vickers M, et al. (2008) Atypical antipsychotic drugs induce
778 derangements in glucose homeostasis by acutely increasing glucagon secretion and
779 hepatic glucose output in the rat. *Diabetologia* 51(12): 2309-2317.
780 Stahl SM, Mignon L and Meyer JM. (2009) Which comes first: atypical antipsychotic
781 treatment or cardiometabolic risk? *Acta Psychiatrica Scandinavica* 119(3): 171-179.
782 Steinert RE, Beglinger C and Langhans W. (2016) Intestinal GLP-1 and satiation: from man
783 to rodents and back. *Int J Obes* 40(2): 198-205.
784 Sturis J, Gotfredsen CF, Romer J, et al. (2003) GLP-1 derivative liraglutide in rats with beta-
785 cell deficiencies: influence of metabolic state on beta-cell mass dynamics. *Br J*
786 *Pharmacol* 140(1): 123-132.
787 Tamura K, Minami K, Kudo M, et al. (2015) Liraglutide Improves Pancreatic Beta Cell Mass
788 and Function in Alloxan-Induced Diabetic Mice. *PLoS ONE* 10(5): e0126003.
789 Tulipano G, Rizzetti C, Bianchi I, et al. (2007) Clozapine-induced alteration of glucose
790 homeostasis in the rat: the contribution of hypothalamic-pituitary-adrenal axis
791 activation. *Neuroendocrinology* 85(2): 61-70.
792 van Can J, Sloth B, Jensen CB, et al. (2014) Effects of the once-daily GLP-1 analog
793 liraglutide on gastric emptying, glycemic parameters, appetite and energy metabolism
794 in obese, non-diabetic adults. *Int J Obes (Lond)* 38(6): 784-793.
795 Weston-Green K, Huang X-F and Deng C. (2011) Olanzapine treatment and metabolic
796 dysfunction: a dose response study in female Sprague Dawley rats. *Behavioural Brain*
797 *Research* 217(2): 337-346.
798 Weston-Green K, Huang X-F and Deng C. (2013) Second generation antipsychotic-induced
799 type 2 diabetes: a role for the muscarinic M3 receptor. *CNS Drugs* 27(12): 1069-1080.
800 Weston-Green K, Huang X-F, Lian J, et al. (2012) Effects of olanzapine on muscarinic M3
801 receptor binding density in the brain relates to weight gain, plasma insulin and
802 metabolic hormone levels. *European Neuropsychopharmacology* 22(5): 364-373.
803 Woodward ND, Purdon SE, Meltzer HY, et al. (2007) A meta-analysis of cognitive change
804 with haloperidol in clinical trials of atypical antipsychotics: Dose effects and
805 comparison to practice effects. *Schizophrenia Research* 89(1-3): 211-224.

806

807 **9. Table and Figure Legends:**

808 **Figure 1** (A) The experimental timeline, (**B**) a schematic representation of the Novel Object
809 Recognition (NOR) test used to assess recognition memory, and **(C)** a schematic
810 representation of the rewarded T-maze alternation test used to assess working memory.

811

812 **Table 1** Fat pad mass (g) of female Sprague Dawley rats following 6-weeks treatment with
813 liraglutide (0.2mg/kg, Lira), olanzapine (2mg/kg, Olan), olanzapine+liraglutide co-treatment
814 (Olan + Lira), clozapine (12mg/kg, Cloz), clozapine+liraglutide co-treatment (Cloz + Lira) or
815 vehicle (Control). Data presented as mean ± S.E.M. (n=9-12/group). *p<0.05 vs. Control,
816 **p<0.01 vs. Control, $^{\#}p$<0.05 vs. Olan, $^{\#\#}p$<0.01 vs. Olan, $^{\#\#\#}p$<0.001 vs. Olan, ^p<0.05 vs.
817 Cloz, ^^p<0.01 vs. Cloz, ^^^p<0.001 vs. Cloz. BAT: Brown adipose tissue.

818

819 **Figure 2** Cumulative **(A)** body weight gain (g), (**B**) food intake (g) and **(C)** water intake (g)
820 in female Sprague Dawley rats following 6-weeks treatment with liraglutide (0.2mg/kg, Lira),
821 olanzapine (2mg/kg, Olan), olanzapine+liraglutide co-treatment (Olan + Lira), clozapine
822 (12mg/kg, Cloz), clozapine+liraglutide co-treatment (Cloz + Lira) or vehicle (Control). Data
823 presented as mean ± S.E.M. (n=10-12/group). *p<0.05 vs. Control, **p<0.01 vs. Control,
824 ***p<0.001 vs. Control, $^{\#\#}p$<0.01 vs. Olan, $^{\#\#\#}p$<0.001 vs. Olan, ^p<0.05 vs. Cloz, ^^p<0.01
825 vs. Cloz.

826

827 **Figure 3 (A)** Blood glucose concentration (mmol/L) and (**B**) glucose Area Under the Curve
828 during an Oral Glucose Tolerance Test in female Sprague Dawley rats following 2-weeks
829 treatment with liraglutide (0.2mg/kg, Lira), olanzapine (2mg/kg, Olan),
830 olanzapine+liraglutide co-treatment (Olan + Lira), clozapine (12mg/kg, Cloz),
831 clozapine+liraglutide co-treatment (Cloz + Lira) or vehicle (Control). Data presented as mean

± S.E.M. (n=10-12/group). *p<0.05 vs. Control, **p<0.01 vs. Control, ***p<0.001 vs. Control, ^^p<0.01 vs. Cloz, ^^^p<0.001 vs. Cloz.

Figure 4 Voluntary locomotor activity showing **(A)** Total distance travelled (m), **(B)** total velocity (m/min) and **(C-D)** hourly distance travelled by female Sprague Dawley rats over 24 hours following 6-weeks of treatment with liraglutide (0.2mg/kg, Lira), olanzapine (2mg/kg, Olan), olanzapine+liraglutide co-treatment (Olan + Lira), clozapine (12mg/kg, Cloz), clozapine+liraglutide co-treatment (Cloz + Lira) or vehicle (Control). Olanzapine and clozapine treatments were administered at 6, 14 and 22 h, while liraglutide was administered at 6 and 22 h. Data presented as mean ± S.E.M. (n=10-12/group). *p<0.05 vs. Control, **p<0.01 vs. Control, ***p<0.001 vs. Control, $^{\#}p$<0.05 vs. Olan.

Figure 5 **(A)** Discrimination ratio and **(B)** total interaction time (s) during a Novel Object Recognition (NOR) test in female Sprague Dawley rats following treatment with liraglutide (0.2mg/kg, Lira), olanzapine (2mg/kg, Olan), olanzapine+liraglutide co-treatment (Olan + Lira), clozapine (12mg/kg, Cloz), clozapine+liraglutide co-treatment (Cloz + Lira) or vehicle (Control). **(C)** Correlation between Discrimination Ratio and total white fat mass (g). Data presented as mean ± S.E.M. (n=11-12/group). ***p<0.001 vs. Control, $^{\#\#\#}p$<0.001 vs. Olan, ^^^p<0.001 vs. Cloz.

Figure 6 Percentage of correct entries in the T-Maze test by female Sprague Dawley rats following 6-weeks treatment with liraglutide (0.2mg/kg, Lira), olanzapine (2mg/kg, Olan), olanzapine+liraglutide co-treatment (Olan + Lira), clozapine (12mg/kg, Cloz), clozapine+liraglutide co-treatment (Cloz + Lira) or vehicle (Control). Data presented as mean ± S.E.M. (n=11-12/group). *p<0.05 vs. Control.

857 Table 1

	Control	Lira	Olan	Olan + Lira	Cloz	Cloz + Lira
Fat Pad Mass (g)						
Inguinal (subcutaneous)	2.2 ± 0.3	**1.3** ± 0.1*	2.8 ± 0.3	**1.8** ± 0.2$^{\#}$	2.3 ± 0.2	1.5 ± 0.1
Perirenal (intra-abdominal)	3.0 ± 0.4	**1.2** ± 0.1*	4.1 ± 0.4	**1.8** ± 0.3$^{\#\#\#}$	3.5 ± 0.4	**1.3** ± 0.1**^^^
Periovary (intra-abdominal)	3.7 ± 0.5	**1.6** ± 0.3**	**5.4** ± 0.6*	**2.3** ± 0.4$^{\#\#\#}$	3.7 ± 0.4	**1.8** ± 0.2**^
Subscapula (BAT)	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
Total White Fat	8.3 ± 1.1	**4.6** ± 0.6**	**12.3** ± 1.0**	**6.0** ± 0.8$^{\#\#\#}$	9.5 ± 0.8	**4.6** ± 0.4**^^

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. control,

# $p < 0.05$, ## $p < 0.01$, ### $p < 0.01$ vs. olanzapine,

^ $p < 0.05$, ^^ $p < 0.01$, ^^^ $p < 0.001$ vs. clozapine.

Figure 1

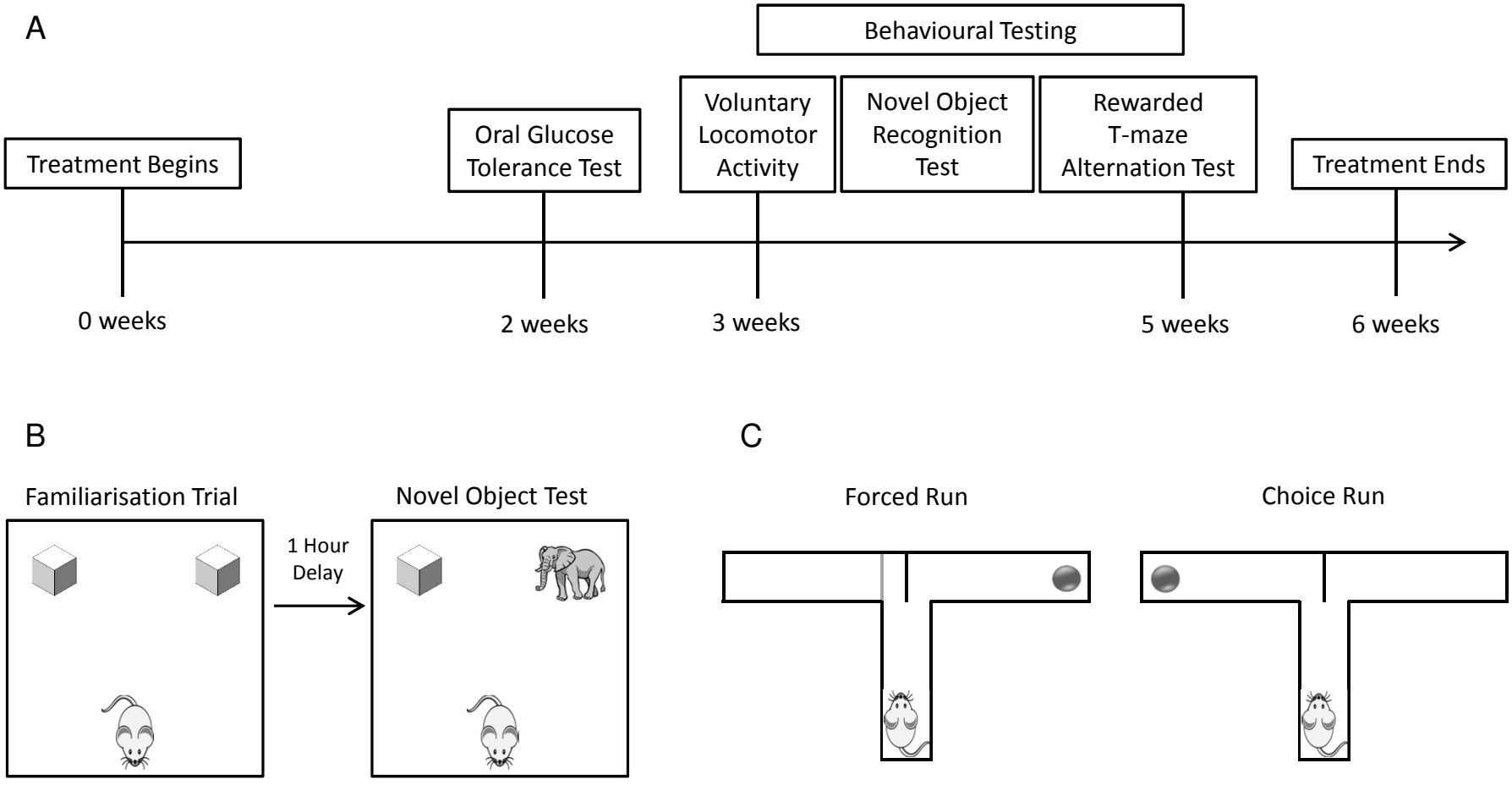

A
Behavioural Testing
Treatment Begins
Oral Glucose Tolerance Test
Voluntary Locomotor Activity
Novel Object Recognition Test
Rewarded T-maze Alternation Test
Treatment Ends
0 weeks
2 weeks
3 weeks
5 weeks
6 weeks
B
Familiarisation Trial
1 Hour Delay
Novel Object Test
C
Forced Run
Choice Run

Figure 2

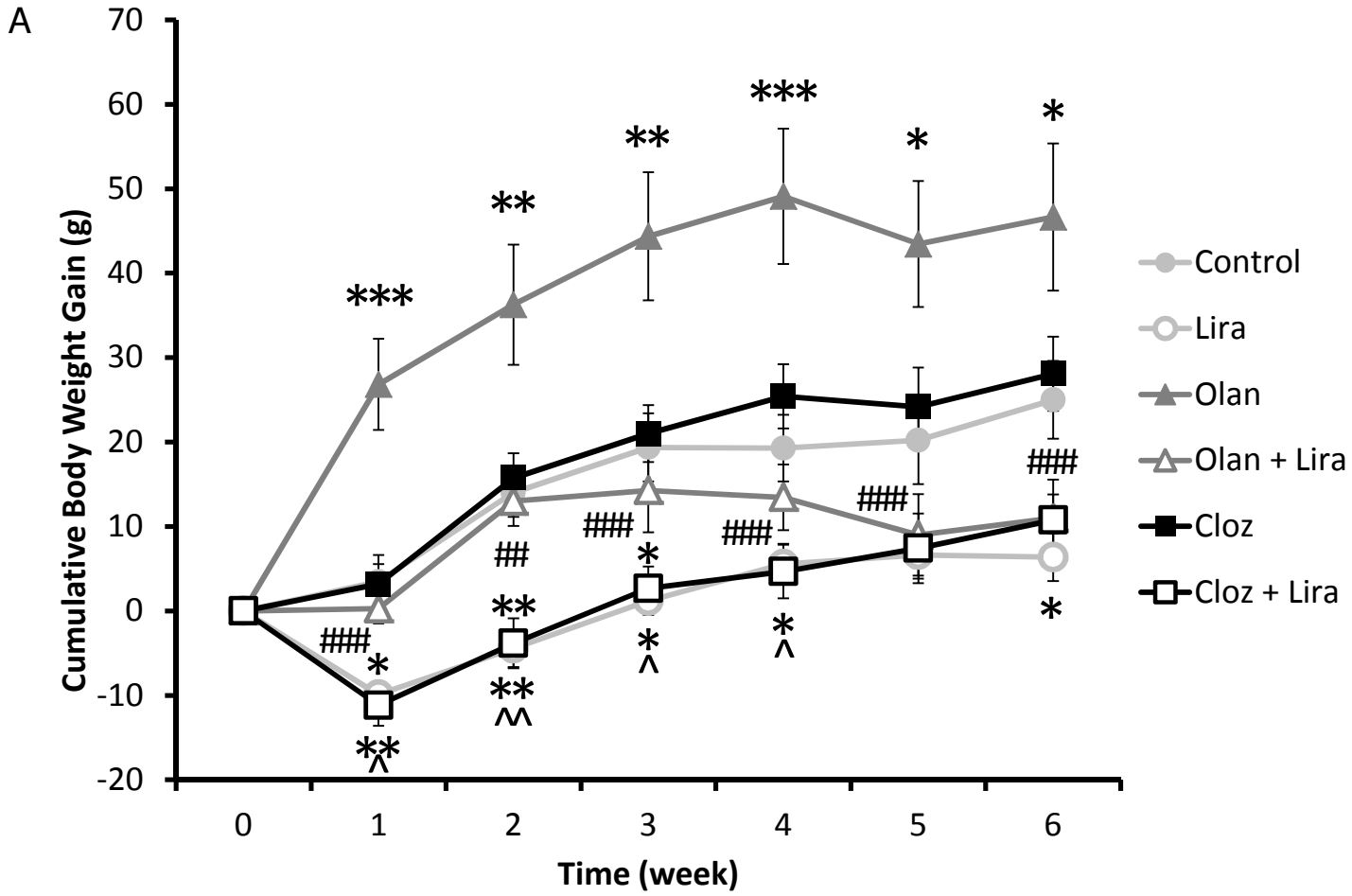

A
Cumulative Body Weight Gain (g)
70
60
50
40
30
20
10
0
-10
-20
0
1
2
3
4
5
6
Time (week)
Control
Lira
Olan
Olan + Lira
Cloz
Cloz + Lira

Figure 2 continued

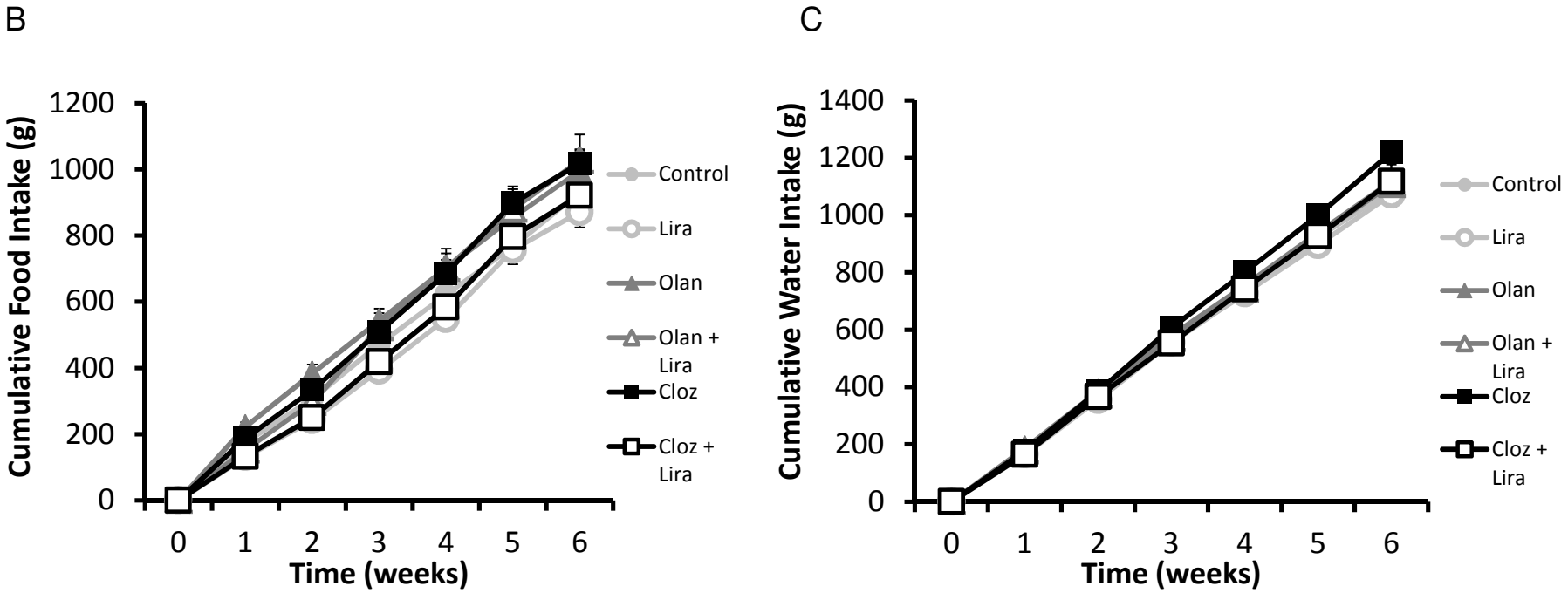


Figure 3

A

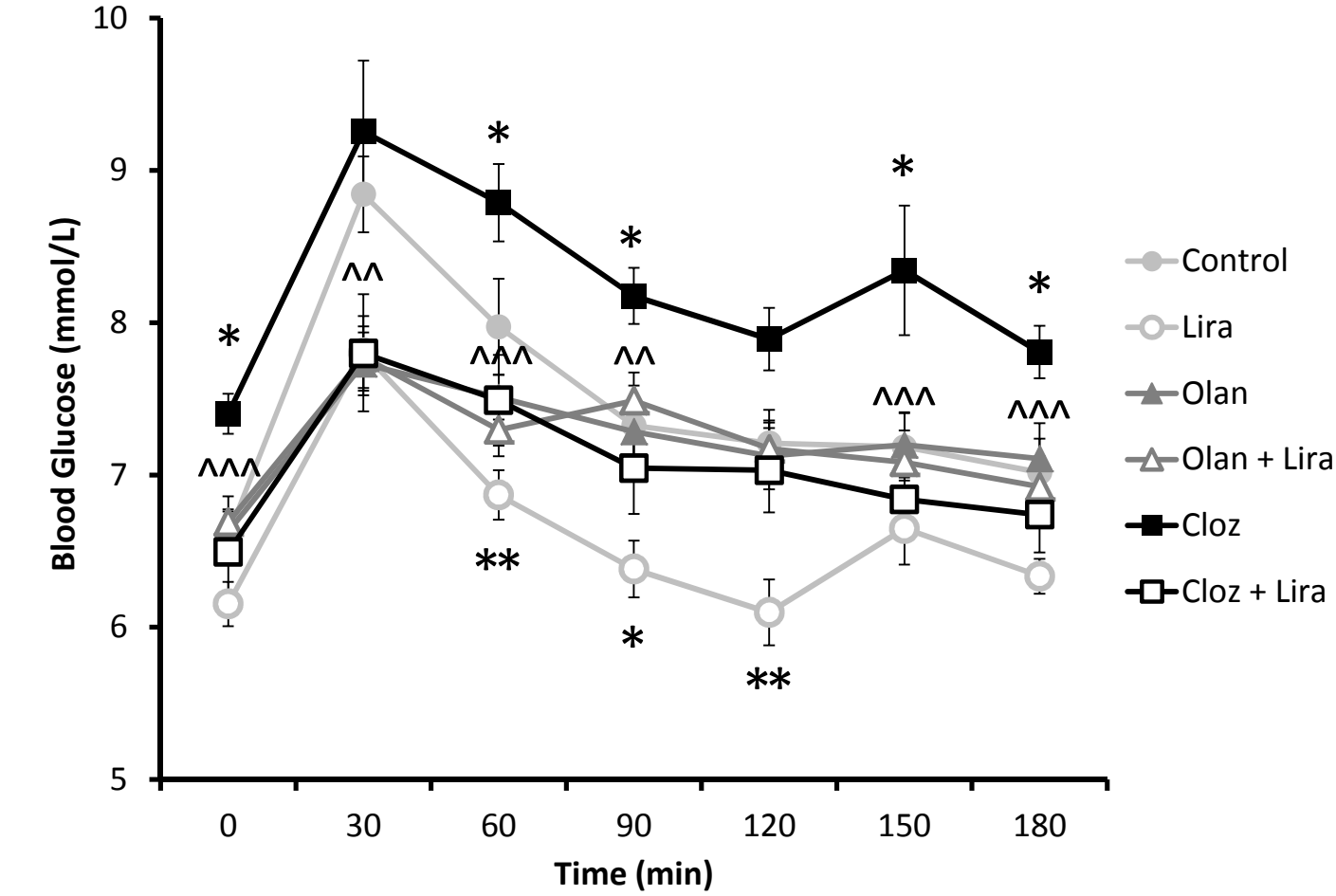

10
9
8
7
6
5
Blood Glucose (mmol/L)
0
30
60
90
120
150
180
Time (min)
Control
Lira
Olan
Olan + Lira
Cloz
Cloz + Lira

Figure 3 continued

B

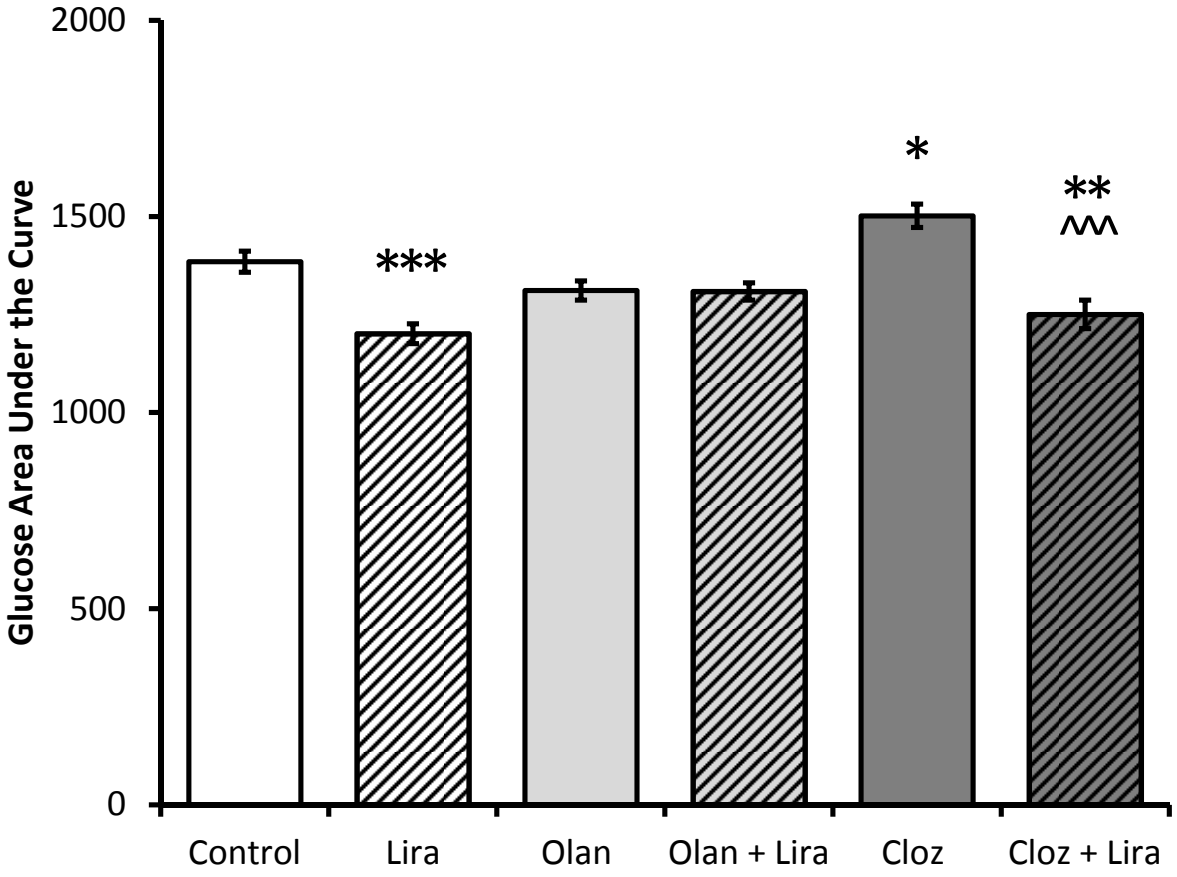


Figure 4

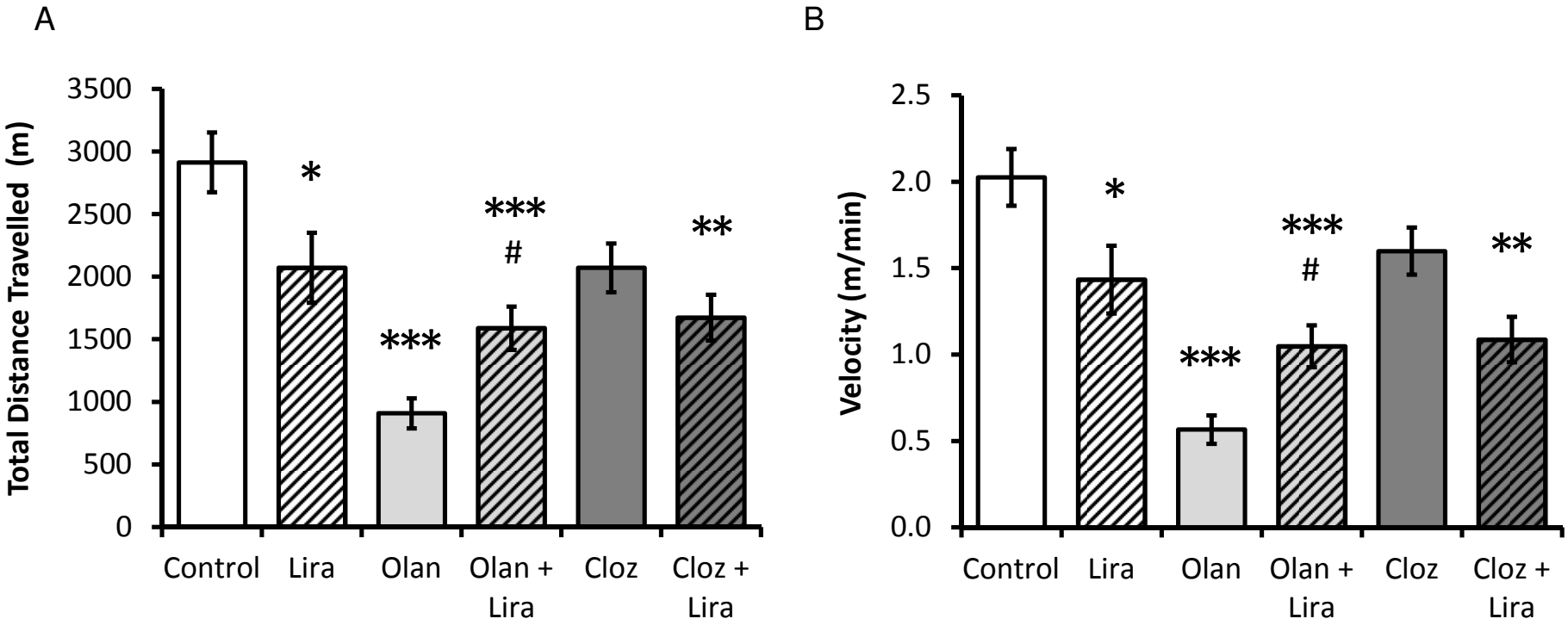

A
Total Distance Travelled (m)
3500
3000
2500
2000
1500
1000
500
0
*
***
#
***
**
Control
Lira
Olan
Olan + Lira
Cloz
Cloz + Lira
B
Velocity (m/min)
2.5
2.0
1.5
1.0
0.5
0.0
*
***
#
***
**
Control
Lira
Olan
Olan + Lira
Cloz
Cloz + Lira

Figure 4 continued

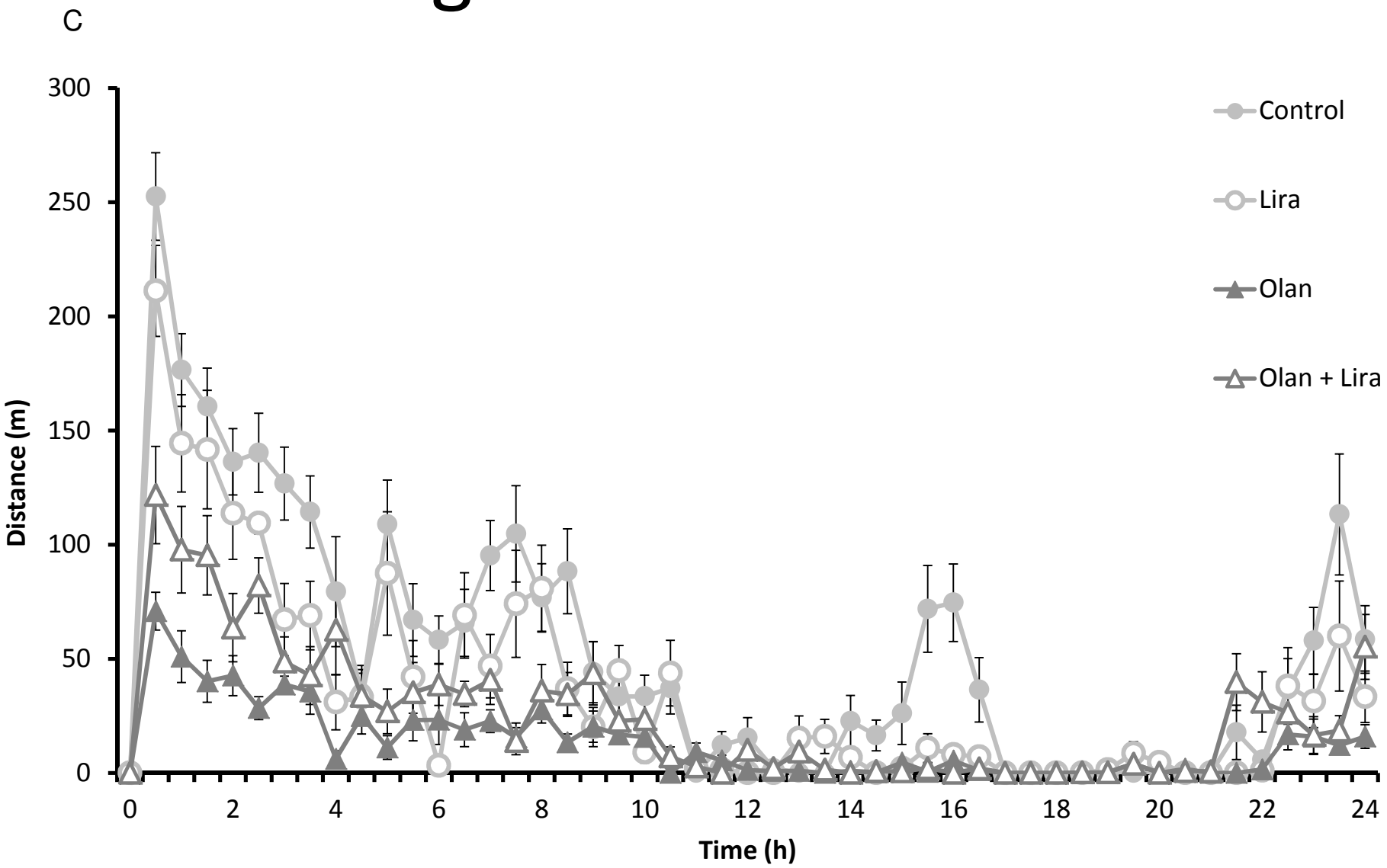
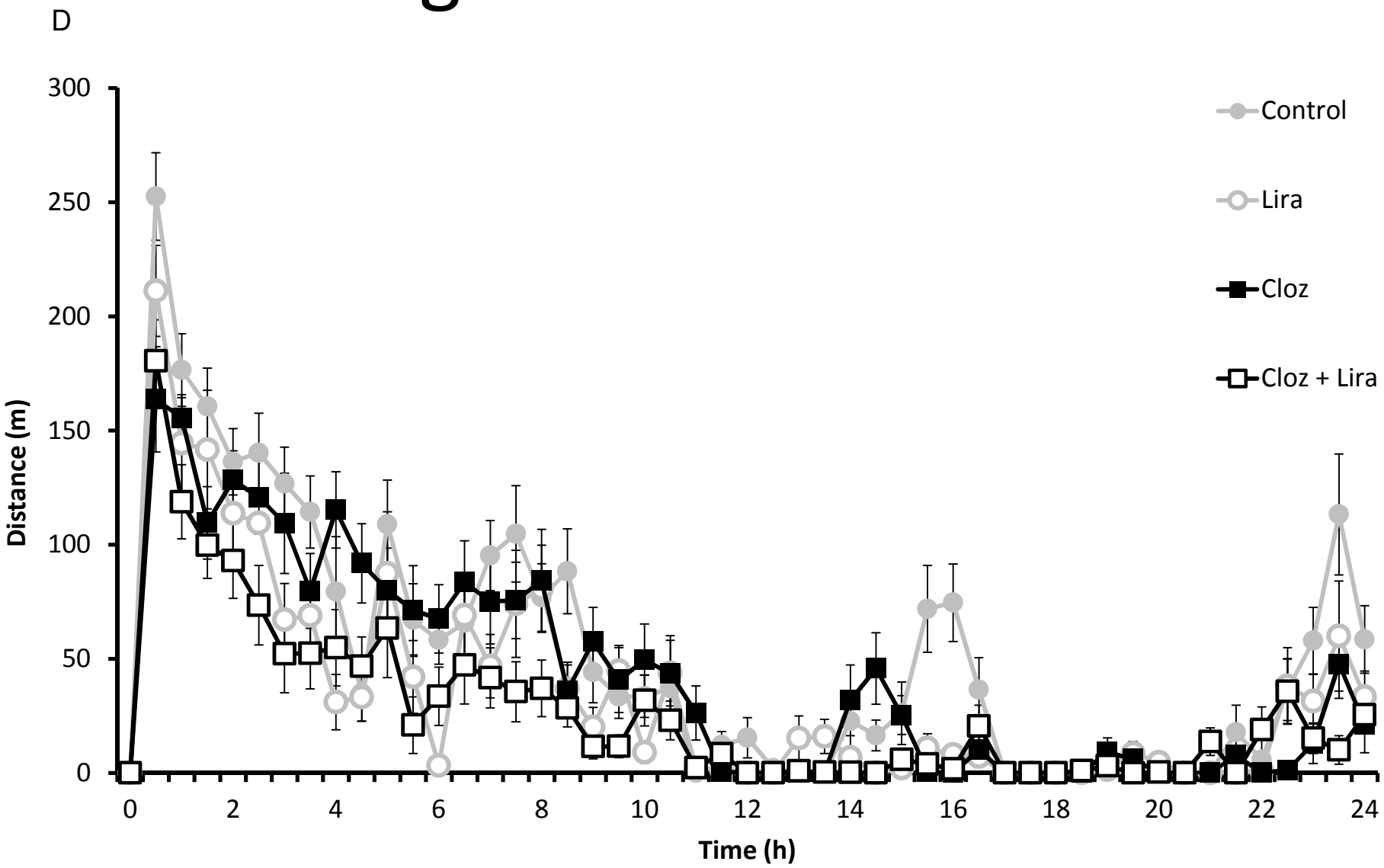


Figure 4 continued



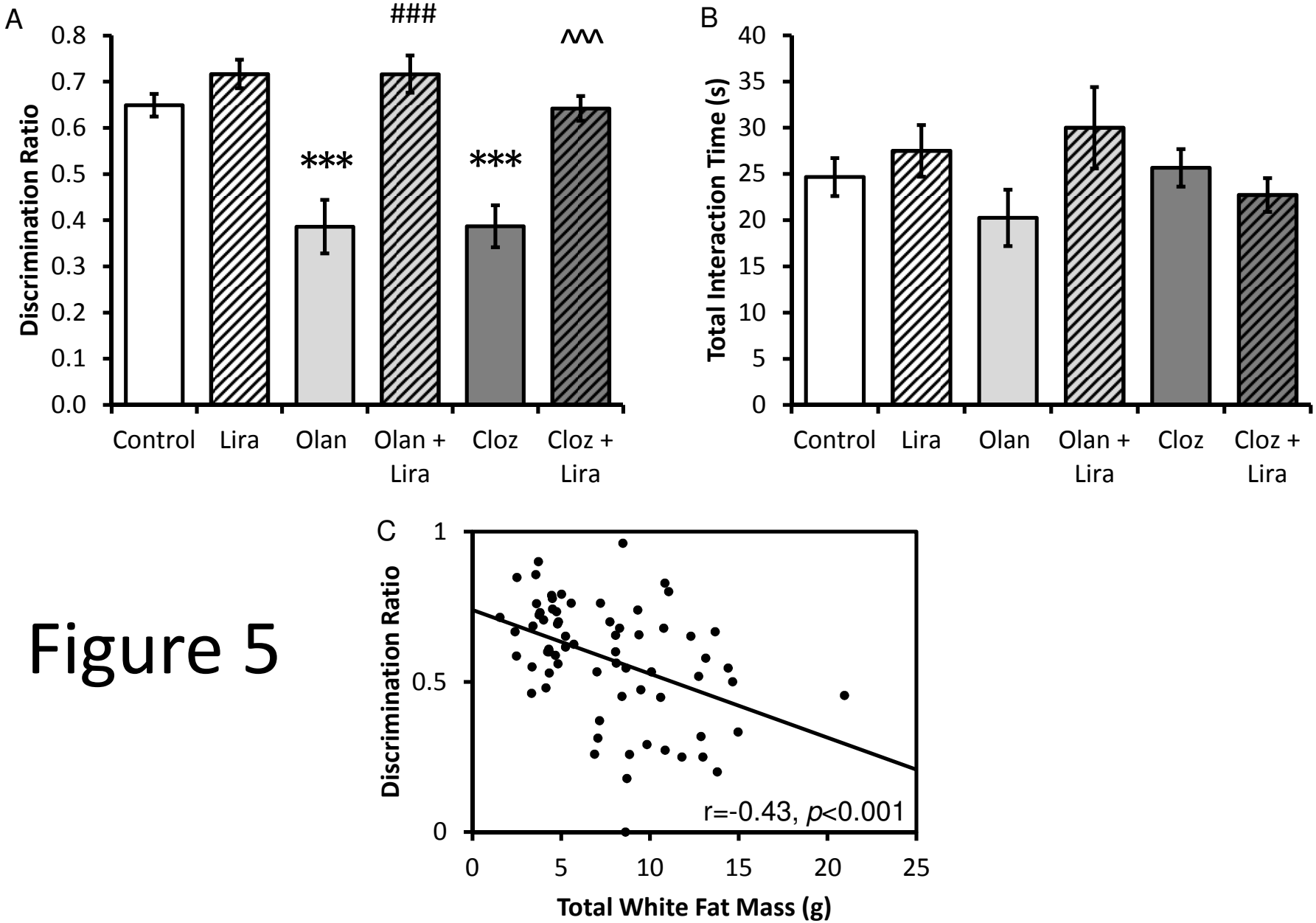
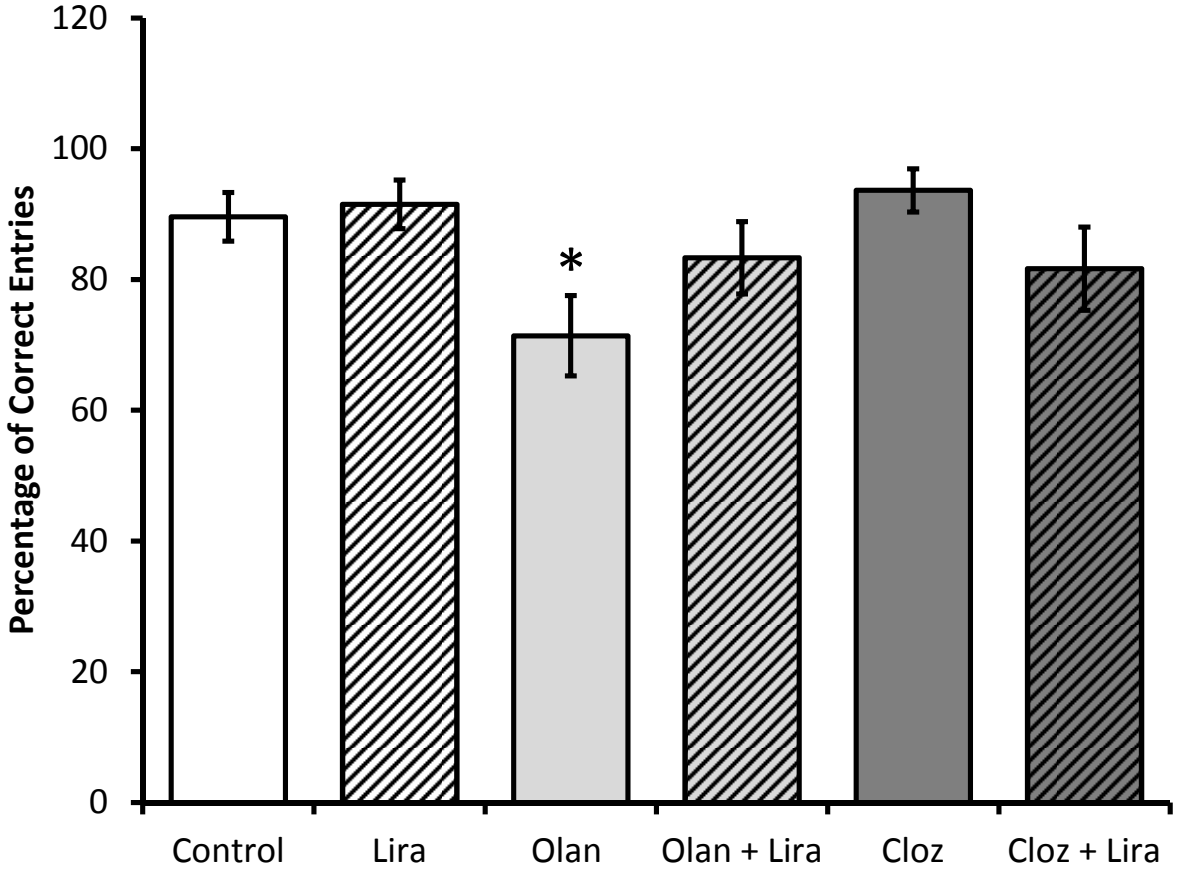

A
Discrimination Ratio
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
###
^^
***
***
Control
Lira
Olan
Olan + Lira
Cloz
Cloz + Lira
B
Total Interaction Time (s)
40
35
30
25
20
15
10
5
0
Control
Lira
Olan
Olan + Lira
Cloz
Cloz + Lira
C
Discrimination Ratio
1
0.5
0
r=-0.43, p<0.001
0
5
10
15
20
25
Total White Fat Mass (g)

Figure 5

Figure 6


120
100
80
60
40
20
0
Percentage of Correct Entries
*
Control
Lira
Olan
Olan + Lira
Cloz
Cloz + Lira