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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

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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

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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

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

Affiliations: \textsuperscript{a} Centre for Medical and Molecular Bioscience, Faculty of Science, Medicine and Health, University of Wollongong NSW Australia 2522, \textsuperscript{b} Illawarra Health and Medical Research Institute, Wollongong, NSW Australia 2522, \textsuperscript{c} Illawarra and Shoalhaven Local Health District, Wollongong, NSW Australia 2500, \textsuperscript{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
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.
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;
Leucht et al., 2009). Furthermore, T2DM and obesity can exacerbate cognitive impairment in schizophrenia patients (Han et al., 2013; Lindenmayer et al., 2012). Overall, novel therapeutic approaches for the treatment of cognitive impairment in schizophrenia and the prevention of metabolic side-effects associated with current medications are required.

Synthetic analogues of the incretin hormone glucagon-like peptide-1 (GLP-1), such as liraglutide and exenatide, can attenuate hyperglycaemia in T2DM and reduce diabetes-associated weight gain (Garber et al., 2011; Robinson et al., 2013; de Wit et al., 2014). Several studies suggest that liraglutide can improve metabolic parameters during APD treatment. For example, a liraglutide increased glucose tolerance and reduced body weight during a recent clinical trial of overweight pre-diabetic individuals schizophrenia spectrum disorder treated with olanzapine or clozapine (Larsen et al., 2017). Liraglutide also induced weight loss and restored glucose homeostasis in an earlier case study of a schizophrenia patient treated with clozapine (Ishøy et al., 2014), and in rats with established obesity and T2DM caused by chronic olanzapine administration (Lykkegaard et al., 2008). Therefore, there is evidence to suggest that liraglutide can treat established metabolic side-effects of olanzapine and clozapine; however, it is unclear whether liraglutide can prevent the onset of weight gain and glucose imbalance when combined with APDs from the start of treatment. In addition to its metabolic benefits, the GLP-1 signalling system in the brain plays an important role in learning, memory and neuroprotection (see reviews by Holscher, 2013; Holst et al., 2011). Endogenous GLP-1 is expressed in the nucleus of the solitary tract (NST) that project throughout the brain (Hamilton and Holscher, 2009). Exogenous glucagon-like peptide-1 receptor (GLP-1R) agonists, liraglutide, lixisenatide and exendin-4, can exert central effects by rapidly crossing the blood brain barrier (Hunter and Holscher, 2012; Kastin and 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
administered twice daily (b.i.d) (0700 and 1500 h), based on the pharmacokinetic half-life of these drugs in the rat (Aravagiri et al., 1999; Baldessarini et al., 1993; Sturis et al., 2003). Cookie dough preparation and administration were conducted as we have previously described (Weston-Green et al., 2011). Body weight, food and water intake were measured weekly. Food intake was measured by weighing the amount of chow remaining in the hopper, including any chow dispersed throughout the cage floor.

2.3 Oral Glucose Tolerance Test (OGTT)

An OGTT was performed during week 2 of treatment. Rats were fasted overnight then fasting (baseline, time 0) blood glucose levels were measured using samples obtained from the lateral tail vein. Measurements were obtained using a handheld glucometer (Accu-Chek Performa Blood Glucose Meter, Sydney, Australia) in triplicate. Rats then received an oral bolus of 2 g/kg glucose and blood sampling was repeated at 30 minute intervals until 180 mins after glucose administration. The mean blood glucose area under the curve (AUC) OGTT blood glucose concentration was calculated using the trapezoidal method, as previously described (Purves, 1992). Treatment groups were alternated throughout the testing period.

2.4 Behavioural Testing

Behavioural testing was performed during weeks 3-5 of treatment to assess 24-hour voluntary locomotor activity, memory and learning. Testing was conducted during the active dark phase (0900-1400 hours) with minimal white light interference and a 24-hour rest period between tests to minimise animal stress. Rats were tested from alternating treatment groups throughout the testing period. Rat behaviour was recorded using standard commercial 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 $\frac{T_N}{T_{TOT}}$ ($T_N$ = 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 CO₂ asphyxiation. Subcutaneous inguinal, intra-abdominal perirenal and periovary white fat pads, and subscapula 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 (perireanal 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 \( (p<0.05 \text{ vs controls, Figure 3B}) \), and failed to reach control levels even after 180 minutes \( (p<0.05 \text{ vs controls, Figure 3A}) \). On the contrary, liraglutide co-treatment prevented the hyperglycaemia caused by clozapine \( (p<0.001 \text{ cloz+lira vs cloz, Figure 3B}) \) and control levels were reached by the end of the test (Figure 3A). Liraglutide treatment significantly decreased the glucose area under the curve \( (p<0.001 \text{ vs controls}) \) (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
The results of the present study show that liraglutide can prevent the obesity side-effects of olanzapine and the chronic hyperglycaemia side-effects of clozapine when administered from the start of treatment. A recent randomised clinical trial of schizophrenia patients treated with either olanzapine (n=15) or clozapine (n=32), who were obese (average patient body mass index of 33) and pre-diabetic found that liraglutide was able to treat already established obesity in these patients (causing an average weight loss of -5.6kg) and improved glycaemic balance by 23% (Larsen et al., 2017). On the other hand, administration of the long acting GLP-1R agonist exenatide (once-weekly) had no effect on body weight in obese schizophrenia patients (Ishøy et al., 2017b). The finding that a liraglutide intervention was able to treat the weight gain and glucose intolerance side-effects of olanzapine was first shown in rodents (Lykkegaard et al., 2008) and in a later case study (Ishøy et al., 2013); however, no studies had examined whether liraglutide could prevent the onset of metabolic side-effects when administered from the start of treatment. Therefore, the results of our study show promise for improved treatment of patients initiating APD treatment who are at an elevated risk of suffering metabolic side-effects.

Liraglutide induces weight loss, in-part, by reducing appetite and inhibitory effects on the reward aspects of feeding behaviour (Holst, 2007; Pi-Sunyer et al., 2015); however, we did not observe significant changes in food intake in treated rats suggesting that body weight alterations were not due to hypophagia and reduced energy intake. Our previous studies showed that olanzapine dramatically increased body weight but with modest increases in food intake (Weston-Green et al., 2011; Weston-Green et al., 2012), demonstrating that appetite did not fully account for the body weight increases. On the contrary, food intake has been found to contribute to olanzapine-induced weight gain in a rat study using a different treatment regime (Davoodi et al., 2009). We also examined voluntary locomotor activity as
reduced motivation towards exercise has been reported in people with schizophrenia prescribed olanzapine and clozapine (Archie et al., 2003; Beebe et al., 2011) and GLP-1R signalling influences mesolimbic reward and motivation pathways in the brain (Richard et al., 2015). Liraglutide co-treatment increased voluntary locomotor activity by 75% compared to the olanzapine group; however, control levels were not restored. Therefore, when considering the basic energy balance equation (energy intake versus energy expenditure) these results suggest that factors other than food intake and exercise are involved in the weight gain caused by olanzapine in the present study, as well as the ability of liraglutide to prevent it. However, pair feeding studies would be required to confirm. Weight changes were associated with increased adiposity in the present study. Indeed, olanzapine promotes lipid accumulation by upregulating the lipogenesis pathway (Albaugh et al., 2010), while liraglutide reduces adipogenic and lipolytic markers (El Bekay et al., 2016). Liraglutide also reduced adiposity below control levels when combined with clozapine, but food intake and locomotor activity were unaltered in the clozapine groups. This is consistent with a previous report that liraglutide lowers the respiratory quotient in obese people, indicating a switch towards increased fat oxidation (van Can et al., 2014). Future investigation into treatment effects on markers of lipid oxidation is warranted.

In the present study, cumulative body weight gain significantly increased with olanzapine, but not clozapine treatment compared to the controls, coinciding with evidence over the past 14 years that rodents are resistant to clozapine-induced weight gain (Choi et al., 2007; Cooper et al., 2008; Albaugh et al., 2006; Pouzet et al., 2003; Weston-Green et al., 2011). Nonetheless, clozapine-induced hyperglycaemia side-effects can be modelled in rodents (Tulipano et al., 2007); a result that was replicated in this study. Similar to our study, the 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
administered alone, but was able to prevent APD-induced cognitive deficits with results demonstrating control-like levels of performance in the co-treatment groups. The inability to discriminate familiar compared to novel objects demonstrates a deficit in recognition memory and learning (Bevins and Besheer, 2006), while impaired T-maze performance suggests impaired working memory (Deacon and Rawlins, 2006), both of which are prominent features of cognitive impairment in schizophrenia (McGuire et al., 2013). A recent clinical study reported no cognitive benefits of long-acting exenatide (once-weekly) in patients with a criteria of obesity, diagnosed schizophrenia spectrum disorder and a minimum of 3 months of APD treatment (including typical 1st and atypical 2nd generation drugs as well as polypharmacy) (Ishøy et al., 2017a). Several explanations for the confounding results may include 1) a difference in dosage between the studies, 2) findings in healthy rodents may not translate to humans with schizophrenia disorders, or 3) possible pharmacological differences between long-acting exenatide and liraglutide. On the latter two points, the same group also reported no effects of long-acting exenatide on body weight in a similar clinical study (Ishøy et al., 2017b); however, liraglutide induced persistent weight loss and metabolic benefits in an earlier case study of an obese patient with schizophrenia treated with clozapine (Ishøy et al., 2013), a result translated from rats (Lykkegaard et al., 2008). Another explanation may be that our prevention study employed liraglutide co-treatment from the start of APD treatment. Studies show that liraglutide can cross the blood brain barrier, promote neuronal stem cell proliferation and neurogenesis, and provides neuroprotective effects (Hunter and Holscher, 2012), including preserved synaptic number and function, and reduced neuroinflammation (McClean and Hölscher, 2014; McClean et al., 2011). In other studies, liraglutide normalised object recognition in a mouse model of Alzheimer’s disease after 8 months of treatment (McClean et al., 2015), exenatide and exendin-4 improved memory and spatial learning in the Morris Water Maze in models of Alzheimer’s disease and cognitive deficits induced by
impaired central insulin signalling (Bomba et al., 2013; Chen et al., 2012), and recognition memory was improved by liraglutide (0.2mg/kg, b.i.d.) in mice following a 20-week high fat diet (Porter et al., 2010). On the other hand, APDs olanzapine and risperidone exacerbated deficits in spatial working memory and attention during the Oculomotor Delayed Response task (Reilly et al., 2007) and decreased dorsolateral prefrontal cortex activation during motor learning tasks (Keedy et al., 2015) in previously APD-naïve schizophrenia patients after 4-6 weeks of treatment. In rodents, olanzapine impaired working memory during Y-Maze and Eight-Arm Radial Maze tests (Castro et al., 2007; Levin et al., 2005; Ortega-Alvaro et al., 2006), and reduced memory acquisition and retrieval in mice during the modified Elevated Plus Maze task (Mutlu et al., 2011). Clinically, APDs can cause varying degrees of sedation (Leucht et al., 2009) and it is possible that sedation impacted the cognitive data. However, in the present study neither APD affected overall exploration time in the NOR test and clozapine treated animals displayed impaired NOR test performance without exhibiting hypolocomotor activity. In addition, treatment effects on reward and motivation may have also played a role in cognitive test performance in the present study.

The underlying mechanisms by which GLP-1 analogues exert beneficial effects on cognition in the brain remain unclear. Interestingly, studies have shown that olanzapine decreases hippocampal volume (Barr et al., 2013) and lowers hippocampal connectivity (i.e. reduces inhibitory terminal immunodensity) in rats (Ramos-Miguel et al., 2015). On the other hand, neuronal GLP-1 has growth characteristics similar to insulin-like growth factor (see review by Bassil et al., 2014), which may enable it to stimulate neurogenesis and hippocampal plasticity to improve cognition. GLP-1 is also a neurotransmitter (Larsen and Holst, 2005), with GLP-1Rs expressed in regions of the brain that are both implicated in cognition and 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
Therefore, further investigation into key neurotransmitter systems and the role of metabolic dysfunction in the cognitive benefits of liraglutide 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

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7. Conflict of Interests

The authors declare that they have no conflicts of interest.
8. References


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9. Table and Figure Legends:

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

Table 1 Fat pad mass (g) of 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=9-12/group). *p<0.05 vs. Control, **p<0.01 vs. Control, ***p<0.001 vs. Control, #p<0.05 vs. Olan, ##p<0.01 vs. Olan, ###p<0.001 vs. Olan, ^p<0.05 vs. Cloz, ^^p<0.01 vs. Cloz, ^^^p<0.001 vs. Cloz. BAT: Brown adipose tissue.

Figure 2 Cumulative (A) body weight gain (g), (B) food intake (g) and (C) water intake (g) in 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=10-12/group). *p<0.05 vs. Control, **p<0.01 vs. Control, ***p<0.001 vs. Control, #p<0.05 vs. Olan, ##p<0.01 vs. Olan, ###p<0.001 vs. Olan, ^p<0.05 vs. Cloz, ^^p<0.01 vs. Cloz.

Figure 3 (A) Blood glucose concentration (mmol/L) and (B) glucose Area Under the Curve during an Oral Glucose Tolerance Test in female Sprague Dawley rats following 2-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=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.
<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Lira</th>
<th>Olan</th>
<th>Olan + Lira</th>
<th>Cloz</th>
<th>Cloz + Lira</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fat Pad Mass (g)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inguinal (subcutaneous)</td>
<td>2.2 ± 0.3</td>
<td><strong>1.3 ± 0.1</strong>*</td>
<td>2.8 ± 0.3</td>
<td><strong>1.8 ± 0.2</strong>*</td>
<td>2.3 ± 0.2</td>
<td>1.5 ± 0.1</td>
</tr>
<tr>
<td>Perirenal (intra-abdominal)</td>
<td>3.0 ± 0.4</td>
<td><strong>1.2 ± 0.1</strong>*</td>
<td>4.1 ± 0.4</td>
<td><strong>1.8 ± 0.3</strong>*</td>
<td>3.5 ± 0.4</td>
<td><strong>1.3 ± 0.1</strong>*^***^</td>
</tr>
<tr>
<td>Periovary (intra-abdominal)</td>
<td>3.7 ± 0.5</td>
<td><strong>1.6 ± 0.3</strong>*</td>
<td><strong>5.4 ± 0.6</strong>*</td>
<td><strong>2.3 ± 0.4</strong>*</td>
<td>3.7 ± 0.4</td>
<td><strong>1.8 ± 0.2</strong>*^***^</td>
</tr>
<tr>
<td>Subscapula (BAT)</td>
<td>0.3 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td>0.3 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td><strong>Total White Fat</strong></td>
<td><strong>8.3 ± 1.1</strong></td>
<td><strong>4.6 ± 0.6</strong>*</td>
<td><strong>12.3 ± 1.0</strong>*</td>
<td><strong>6.0 ± 0.8</strong>*</td>
<td><strong>9.5 ± 0.8</strong></td>
<td><strong>4.6 ± 0.4</strong>*^***^</td>
</tr>
</tbody>
</table>

* *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

<table>
<thead>
<tr>
<th>Event</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment Begins</td>
<td>0 weeks</td>
</tr>
<tr>
<td>Oral Glucose Tolerance Test</td>
<td>2 weeks</td>
</tr>
<tr>
<td>Voluntary Locomotor Activity</td>
<td>3 weeks</td>
</tr>
<tr>
<td>Novel Object Recognition Test</td>
<td>3 weeks</td>
</tr>
<tr>
<td>Rewarded T-maze Alternation Test</td>
<td>5 weeks</td>
</tr>
<tr>
<td>Treatment Ends</td>
<td>6 weeks</td>
</tr>
</tbody>
</table>

B

1 Hour Delay

Familiarisation Trial → Novel Object Test

C

Forced Run

Choice Run
Figure 2
Figure 2 continued

B

C

Cumulative Food Intake (g)

Cumulative Water Intake (g)

Time (weeks)

Control
Lira
Olan
Olan + Lira
Cloz
Cloz + Lira

Time (weeks)

Control
Lira
Olan
Olan + Lira
Cloz
Cloz + Lira
Figure 3

A graph showing the blood glucose levels over time for different groups. The x-axis represents time in minutes (0, 30, 60, 90, 120, 150, 180), and the y-axis represents blood glucose levels in mmol/L. The groups are labeled as Control, Lira, Olan, Olan + Lira, Cloz, and Cloz + Lira. The graph includes asterisks and other symbols to indicate statistical significance.
Figure 3 continued

B

Glucose Area Under the Curve

Control  Lira  Olan  Olan + Lira  Cloz  Cloz + Lira

0  500  1000  1500  2000
Figure 4

A

Total Distance Travelled (m)

Control  Lira  Olan  Olan + Lira  Cloz  Cloz + Lira

B

Velocity (m/min)

Control  Lira  Olan  Olan + Lira  Cloz  Cloz + Lira

*  ***  #  **
Figure 4 continued

- Control
- Lira
- Olan
- Olan + Lira

Distance (m) vs Time (h)
Figure 4 continued

- Control
- Lira
- Cloz
- Cloz + Lira

Distance (m) vs. Time (h) with error bars. The graph shows the movement over time, with different treatments (Control, Lira, Cloz, Cloz + Lira) indicated by various symbols and colors.
Figure 5

**A**  
Discrimination Ratio

- Control
- Lira
- Olan
- Olan + Lira
- Cloz
- Cloz + Lira

**B**  
Total Interaction Time (s)

- Control
- Lira
- Olan
- Olan + Lira
- Cloz
- Cloz + Lira

**C**  
Discrimination Ratio vs. Total White Fat Mass (g)

- $r = -0.43$, $p < 0.001$
Figure 6

Percentage of Correct Entries

Control  Lira  Olan  Olan + Lira  Cloz  Cloz + Lira

*