EFFECTS OF ANTIPSYCHOTICS AND MOOD STABILIZERS ON MITOCHONDRIAL MORPHOLOGY
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INTRODUCTION
Mitochondrial dysfunction has been suggested to play a critical role in the pathophysiology of bipolar disorder and schizophrenia (Quiror et al., 2008; Kato et al., 2000), and schizophrenia (Jensen et al., 2006; Jayakumar et al., 2006). Although produced by active mitochondria during oxidative phosphorylation, an over accumulation of reactive oxygen species (ROS) can overwhelm the antioxidant capacity of the cell, thereby causing cell damage or death (Johannsen et al., 2009). Mitochondrial dysfunction is associated with increased levels of ROS (Johannsen et al., 2009) - which has also been associated with the use of atypical antipsychotics such as Clozapine. We have previously found that Clozapine induces swelling of mitochondria, which is an indication of mitochondrial dysfunction (Waiss-Bass et al., 2008). We have also shown that the newly approved atypical antipsychotic paliperidone has a mode of action similar to lithium and valproic acid, sharing several common pathways especially (Corena-McLeod et al. 2008). In this study we investigated the effect of Paliperidone, Clozapine and Lithium on mitochondrial morphology in the human neuroblastoma cell line SK-N-SH, in an attempt to elucidate the molecular pathways by which these drugs cause their therapeutic effects.

METHODS
Cell culture
Human neuroblastoma cells (SK-N-SH) were maintained in Minimum Essential Medium (MEM) supplemented with 10% heat-inactivated Fetal Bovine Serum, penicillin (45U/ml), streptomycin (45µg/ml), MEM Non-Essential Amino Acids (90µK), and pyruvic acid (90µM) in plastic Corning flasks at 37°C under 5% CO2/95% air. Medium was changed 3 times/wk.

Drug treatment
Confluent cultures were washed with phosphate-buffered saline (PBS), followed by treatment with clozapine (10, 20 or 50 µM), lithium (1, 10 or 20 µM), paliperidone (1, 10 or 50 µM) or vehicle for 24 h. Experiments were performed in triplicate with all drug concentrations. Cells were seeded onto 8-well chambered cover glasses.

Confocal Imaging
To visualize mitochondrial morphology, cells were incubated with 100 nM MitoTracker® Green for 1 hr at 37°C under 5% CO2/95% air (Invitrogen, Eugene, OR, USA). Confocal images were obtained using a FV1000 imaging system (Olympus America, Center Valley, PA, USA) mounted on an Olympus IX-81 research grade microscope. The lens used for imaging was a PlanApo 60X (Olympus America, Center Valley, PA, USA), NA 1.4, oil immersion. Acquisition conditions were determined for each imaging channel using individually labeled fluorescent samples.

RESULTS
Our results indicate that treatment with clozapine resulted in changes in mitochondrial morphology, compared to those observed after paliperidone and lithium treatment. Clozapine induced swelling of mitochondria, indicative of active oxidation, and relocation of mitochondria in a network around the nucleus. Paliperidone and lithium did not result in increased mitochondrial volume but appeared to cause changes in mitochondrial movement and activity as indicated by the enhanced appearance of filamentous mitochondria.

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DISCUSSION
The apparent changes in mitochondrial movement and activity induced by lithium, a mood stabilizer, and paliperidone, an atypical antipsychotic, as well as with neuroleptic treatment. We report changes in mitochondrial morphology associated with antipsychotic and mood stabilizer treatment. In this study SKNSH neuroblastoma cells were treated with increasing concentrations of clozapine, paliperidone and lithium for 24 hrs. Mitochondria were visualized by confocal microscopy using mitotracker green. It was observed that clozapine induces swelling of mitochondria, indicative of active oxidation. Paliperidone and lithium did not result in increased mitochondrial volume but appear to cause changes in mitochondrial movement and activity as indicated by the enhanced appearance of filamentous mitochondria. These results suggest that lithium and paliperidone enhance mitochondrial respiration while clozapine decrease it.

DISCLOSURES
Part 3: Financial Involvement with pharmaceutical, biotech, or medical device which constitutes more than 5% of personal income (Jan 2007-Present)
Part 4: Grants from pharmaceutical, biotech, or medical device companies directly, or indirectly through a foundation, university, or any other organization (Jan 2007-Present).

CONCLUSIONS
We have used confocal microscopy to identify changes in mitochondrial morphology associated with antipsychotic and mood stabilizer treatment. Paliperidone, an atypical antipsychotic, does not appear to cause mitochondrial swelling or changes in mitochondrial movement and activity similar to lithium, a mood stabilizer. This suggests that paliperidone has a similar mode of action as lithium and can potentially reverse disease-related alterations in mitochondrial of bipolar patients (Maurer et al 2009). The observed enhancement of swelling of mitochondria may be a mechanism by which these drugs reverse disease-related alterations in mitochondria of bipolar patients (Maurer et al 2009). The observed changes induced by lithium and paliperidone may be a mechanism by which cells prevent oxidative damage due to increased generation of mitochondrial ROS (mROS) by the respiratory chain (Boviers et al, 1972). Previous studies have shown that minor oxidative stress induces the formation of a mitochondrial “firewall” which prevents propagation of mROS (Jou et al 2008).

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