Ketamine role in the treatment of Maternal depression: effects on offspring behaviour

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Abstract
Maternal depression during pregnancy adversely affects offspring neurodevelopment and behaviour. Typical antidepressants like selective serotonin reuptake inhibitors have limitations due to risks of crossing the placenta. Ketamine has emerged as a promising alternative treatment. This research examined ketamine's effects on offspring of maternally stressed mice. Dams were divided into control, maternal adversity, fluoxetine, and ketamine groups. Open field, sucrose preference, elevated plus maze, and forced swim tests assessed offspring anxiety, anhedonia, and despair. Maternal adversity increased anxiety-like behaviours and ketamine or fluoxetine reversed some effects. However, fluoxetine more effectively mitigated despair in forced swim tests. Ketamine moderately alleviated anhedonia versus controls. Further research on dose-response and timing is needed to optimize ketamine treatment. Mitigating maternal depression is crucial for preventing maladaptive offspring neurobehavioral trajectories.

INTRODUCTION
The perinatal period marks a sensitive developmental window where maternal wellbeing exerts profound impacts on foetal and offspring growth (Dean et al. 2018). Maternal stress, anxiety, and depression during pregnancy occur in up to 20% of expecting mothers (Leung and Kaplan 2009) and confer risk for adverse offspring outcomes like depression, attention-deficit hyperactivity disorder (ADHD), and impaired cognition (Dean et al. 2018; Weinstock 2001; Rayen et al. 2013). The precise biological mechanisms linking maternal mood to offspring development involve the hypothalamic-pituitary-adrenal (HPA) axis, placental function, inflammation, and neurotransmitters like serotonin (Weinstock 2001; Czarzasta et al. 2019). Ultimately, these pathways influence gene expression, neural connectivity, and behaviour (Tang et al. 2021; Bashiri et al. 2021; Mbiydzenyuy et al. 2022; Sheng et al. 2021).

Firstly, maternal adversity activates the HPA axis, increasing circulation of corticotrophin releasing hormone (CRH), adrenocorticotropic hormone (ACTH), and cortisol (Weinstock 2001; Amani et al. 2021). Cortisol crosses the placenta, directly impacting the offspring’s developing HPA axis, predisposing them to affective disorders and cognitive deficits (Czarzasta et al. 2019). Fluoxetine treatment during pregnancy prevents some HPA axis alterations by reducing corticosterone and ACTH levels while increasing oxytocin and vasopressin expression (Bashiri et al. 2021).

Additionally, proinflammatory cytokines triggered by stress, anxiety, and depression can cross the placenta, activating microglia and astrocytes in the foetal brain (Dean et al. 2018). This sets in motion inflammatory cascades altering neurotransmitters, neurotrophic factors, hormones, and neural connectivity (Dean et al. 2018). Fluoxetine may mitigate inflammation-induced epigenetic changes, as it increased histone acetylation of brain-derived neurotrophic factor (BDNF) and corticotrophin releasing factor receptor 2 while decreasing methylation of these key genes related to stress resilience (Bashiri et al. 2021).
Serotonin also plays a major role in neurodevelopment, regulating neuronal differentiation, migration, synaptogenesis, and network wiring (Rayen et al. 2013; Oliver et al. 2021). Disrupting the serotonergic system with selective serotonin reuptake inhibitors (SSRIs) like fluoxetine could have unintended consequences on the offspring's brain development and later behaviour (Rayen et al. 2013). For example, developmental fluoxetine exposure decreased size of the sexually dimorphic nucleus preoptic area, tyrosine hydroxylase cell number in the anteroventral periventricular nucleus, and volume of the posterior bed nucleus of the stria terminalis – all regions strongly implicated in social and sexual behaviours (Rayen et al. 2013).

Rodent studies confirm fluoxetine's neurodevelopmental effects, showing reduced ultrasonic vocalizations in pups and social play in adolescents after maternal fluoxetine exposure (Houwing et al. 2019). Male offspring also demonstrated lower social interaction in adulthood (Houwing et al. 2019). Though SSRIs like fluoxetine remain frontline treatments for perinatal depression, their unintended impacts on foetal neurodevelopment highlight the need for alternative interventions that effectively treat mothers without secondary consequences for the next generation.

Ketamine has recently emerged as a promising antidepressant, demonstrating rapid and sustained effects within hours compared to weeks for conventional antidepressants (Loo et al. 2016; Matveychuk et al. 2020; Salvador and Singh 2013; Browne and Lucki 2013). Unlike SSRIs targeting serotonin, ketamine primarily antagonizes N-methyl-D-aspartate (NMDA) glutamate receptors and enhances α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor transmission (Iadarola et al. 2015; Zanos and Gould 2018). This cascade stimulates release of neurotrophic factors like BDNF to promote synaptogenesis and reverse synapse loss associated with depression (Loo et al. 2016). Ketamine's distinct mechanisms could thus treat maternal depression without directly interfering in serotonin-mediated developmental processes.

Indeed, animal studies found that maternal ketamine administration buffered pups against prenatal stress by preventing excessive ultrasonic vocalizations and improving spatial memory (Calpe-López et al. 2022). Another study showed that postnatal ketamine exposure prevented depression-like behaviour in maternally separated rat pups (Fukushima et al. 2022). While early findings in offspring are optimistic, rodent timing does not perfectly translate to human neurodevelopment, so further research is essential to ensure ketamine safety.

Some human trials show promising results as well. A recent randomized control pilot study found that a single sub-anaesthetic ketamine infusion was feasible, well-tolerated, and efficacious for reducing depressive symptoms in the postpartum period (Balachandran et al. 2022; Muscat et al. 2021). In an open-label study of pregnant women with treatment-resistant depression, over half of patients achieved remission sustaining for at least 2 weeks post-infusion (McIntyre et al. 2023; Shiroma et al 2020). Larger controlled trials are currently underway to further investigate maternal ketamine efficacy and safety (Jelovac et al. 2023; Strong and Kabbaj 2018).
This research aimed to compare effects of fluoxetine and ketamine on offspring behavior following exposure to an adversive maternal environment modelled by chronic mild stress. Based on clinical evidence and distinct mechanisms of action, we hypothesized that ketamine could mitigate some deleterious effects of maternal adversity without directly interfering in offspring neurodevelopment like fluoxetine. We specifically examined offspring anxiety, anhedonia, and despair-like behaviour in elevated plus maze, sucrose preference, and forced swim tests respectively. Characterizing impacts on neurobehavioral outcomes is an essential step toward optimizing maternal depression treatment to prevent transgenerational consequences.

MATERIALS AND METHODS

Subjects: Forty albino dam mice approximately 8 weeks old were used, with 10 mice randomly assigned to each of 4 treatment groups: 1) Control with no adversity or medication, 2) Maternal adversity without medication, 3) Maternal adversity with fluoxetine 10 mg/kg, and 4) Maternal adversity with ketamine 5 mg/kg. Litters were culled to 6 pups after birth. At weaning on postnatal day 21, 2–3 male offspring from each litter were housed together throughout behavioural experiments. All procedures were approved by the Institutional Animal Care and Use Committee and kept under standard protocol adopted in previous studies (Faisal et al. 2022; Abdulqader et al. 2022).

Maternal Adversity Paradigm: The maternal adversity protocol was adapted from previous studies exposing rodent dams to chronic variable stress during late pregnancy (Bashiri et al. 2021; Czarzasta et al. 2019). From embryonic days 14–19, dams underwent daily stress exposure consisting of overnight illumination, tilted cages, disrupted light/dark cycles, novel objects, fox odour, noise, and 15 minutes of restraint stress. Control dams remained in normal housing conditions without any stressors.

Drug Administration: Stressed dams received intraperitoneal injections of fluoxetine 10 mg/kg, ketamine 5 mg/kg, or saline vehicle from embryonic days 14–19. The fluoxetine dose was selected based on prior research demonstrating efficacy in mitigating maternal separation effects (Bashiri et al. 2021). The ketamine dose matches common antidepressant ranges without reaching anaesthetic levels (Carrier and Kabbaj 2013). All injections occurred after the daily stress exposure between 5–7 pm.

Behavioural Testing: All assays were conducted on male offspring at 8–10 weeks old. Up to 3 males from each litter were tested as biological replicates. Animals were habituated to the testing room 2 hours prior to experiments.

Elevated Plus Maze: The elevated plus maze assessed anxiety-related behaviours through preferential exploration of open and closed arms. The maze consisted of two open arms without walls and two enclosed arms surrounded by 15 cm high walls, elevated 63 cm above the floor. Arms were 30 cm long and 5 cm wide. The test began by placing mice in the central junction facing an open arm and allowing free exploration for 5 minutes. ANY-maze software tracked movements. Time spent in open versus closed arms was analysed. Increased open arm exploration indicates lower anxiety-like behaviour.
Forced Swim Test: The forced swim test evaluated behavioural despair. Mice were placed for 6 minutes into a transparent cylinder filled with 30 cm deep water, 25–27°C. The test commenced by gently lowering mice into water and videotaping from the side. ANY-maze software scored mobility versus floating, defined as immobility for over 2 seconds. Increased time spent immobile and not attempting escape is indicative of despair-like learned helplessness.

Sucrose Preference: Sucrose preference assessed anhedonia and reward-seeking deficits. Mice were habituated to 1% sucrose solution for 48 hours. Food and water were removed 6 hours before testing. Mice were presented with two identical bottles, one filled with water and one with 1% sucrose solution. Bottles were weighed before and after the 2-hour test. Sucrose preference percentages were calculated from volumes of sucrose solution consumed divided by total volume of sucrose solution plus water consumed. Decreased sucrose preference indicates depression-like anhedonia.

Open Field Test: The open field test evaluated anxiety-like behaviours through exploratory locomotion in a novel environment. The 50cm x 50cm x 50cm box had black plexiglass walls and white flooring. Movements were video recorded for 60 minutes under red lighting and tracked by ANY-maze software. Time spent in the centre zone versus periphery was analysed as a measure of anxiety, along with total distance travelled over time. Increased centre zone exploration and higher locomotors activity suggest lower anxiety.

Statistical Analysis: One-way ANOVAs followed by Dunnett’s multiple comparisons tests evaluated effects between control, maternal adversity, fluoxetine, and ketamine groups. Two-way repeated measures ANOVAs assessed open field effects over time. GraphPad Prism version 8.4.2 was used for all analyses with significance defined as p < 0.05. All data are presented as mean ± SEM.

RESULTS

Locomotors Activity: An open field test evaluated effects on exploratory locomotion over 1 hour (Fig. 1). Overall, distance travelled progressively decreased in all groups, reflecting habituation to the novel environment over time. However, significant differences emerged at specific intervals:

Compared to the maternal adversity group, fluoxetine-treated offspring displayed heightened locomotors activity at 50 and 60 minutes. Fluoxetine also increased activity versus ketamine at 50 minutes. This hyperactive drive may relate to dopaminergic or serotonergic signalling imbalances, potentially indicative of overcompensation effects from developmental SSRI exposure previously shown in rodents.

Elevated Plus Maze: Anxiolytic effects were assessed on the elevated plus maze in Fig. 2. Relative to controls, maternal adversity significantly decreased time spent exploring the open arms, reflecting heightened anxiety. Ketamine and fluoxetine treatments partially normalized this effect, as both groups performed midway between adversity and controls. While increased open arm preference represents reduced anxiety with treatment, the intermediate effect suggests maternal adversity may still confer lingering impacts even with drug interventions in early neurodevelopment.
Sucrose Preference: The sucrose preference test evaluated anhedonia through intake of a rewarding sucrose solution (Fig. 3). Compared to controls, the maternal adversity group showed considerably lower sucrose preference, indicative of a depression-like reward deficit state. Mirroring clinical findings, both fluoxetine and ketamine elicited at least partial restoration of sucrose preference versus maternal adversity. The robust effects align with previous reports of their rapid antidepressant actions, though the lower performance relative to controls warrants further dose optimization.

Forced Swim Test: Finally, a forced swim test assayed behavioural despair phenotypes (Fig. 4). Maternal adversity significantly increased floating time, suggestive of despair-like learned helplessness. Here, in contrast to sucrose tests, ketamine was ineffective while fluoxetine potently decreased immobility versus both control and adversity groups. This more sustained antidepressant-like response may relate to the role of serotonin signalling in long-term stress coping. The findings indicate pathway-specific impacts, with advantages of fluoxetine over ketamine for alleviating despair but potentially at the expense of precarious neurodevelopmental disruption.

DISCUSSION

This study provides evidence that ketamine could effectively treat aspects of maternal adversity-induced depression-like phenotypes in offspring without conferring adverse consequences of direct serotonin disruption like fluoxetine. Both drugs alleviated anxiety-like behaviours in an elevated plus maze and reversed maternal adversity’s impacts on anhedonia based on sucrose preference tests. However, in a forced swim test assessing behavioural despair, fluoxetine outperformed ketamine. These findings have implications for optimizing maternal depression treatment to support healthy neurodevelopment.

Maternal adversity exposure utilizing chronic stressors mimics elements of human environmental risk factors that could act alone or interact with genetic vulnerabilities to induce perinatal depression. As predicted based on prior work, offspring demonstrated anxiety-like behaviour with reduced open arm exploration on the elevated plus maze (Tang et al. 2021; Bashiri et al. 2021) coupled with decreased sucrose preference indicating anhedonia (Dean et al. 2018; Planchez et al. 2019). Open field testing revealed no differences in center zone exploration but did suggest hyperlocomotion in the fluoxetine group.

Both ketamine and fluoxetine ameliorated anxiety-related behaviours, aligning with known anxiolytic effects (Houwing et al. 2019; Carrier and Kabbaj 2013). The mechanism may involve buffering excess stimulation of the HPA axis and downstream corticosterone release triggered by maternal stress (Bashiri et al. 2021). A rat model of inflammation-induced prenatal stress found that fluoxetine prevented epigenetic effects on corticotrophin releasing factor receptor 2, which could mitigate anxiety by potentiating serotonin signalling (Bashiri et al. 2021). In contrast, ketamine may reduce anxiety through opposing interactions with NMDA and AMPA glutamate receptors which regulate excitation/inhibition balance (Carrier and Kabbaj 2013).
Our results also demonstrate ketamine and fluoxetine's efficacy in treating anhedonia, one of depression's hallmark symptoms. This adds to existing evidence that ketamine alleviates reward processing deficits in both human and animal studies (Carrier and Kabbaj 2013; Lally et al. 2015; Lullau et al. 2023). Increased ventral striatal responses to reward cues post-ketamine infusion correlated with reduced anhedonia ratings, suggesting medium spiny neuron disinhibition. Enhancing AMPA throughput could strengthen dopaminergic encoding of value and salience (Francois et al. 2016). Unlike traditional antidepressants targeting the serotonergic system, ketamine may more directly modulate reward circuitry through glutamate and dopamine interactions (Carrier and Kabbaj 2013).

In the forced swim test though, ketamine failed to impact floating time whereas fluoxetine robustly decreased immobility. Our results support previous studies showing limited effects of ketamine on despair-like behaviour in this specific assay, potentially due to stress-induced corticosterone interference (Carrier and Kabbaj 2013; Franceschelli et al. 2015). However clinical investigations demonstrate ketamine rapidly reduces suicidal ideation in treatment-resistant patients, so lack of effects in rodent forced swim may simply reflect a poor model of predictive validity for this singular dimension of depression (Wilkinson and Sanacora 2019). Patients describe the post-ketamine state as no longer feeling burdened by negative thoughts rather than a stimulated mood, so the test may not capture relevant phenomenology (Loo et al. 2016). Regardless, our findings indicate superior efficacy of fluoxetine over ketamine for mitigating behavioural despair in offspring exposed to maternal adversity.

Open field results were more difficult to interpret, with no clear effects on centre zone exploration but seemingly increased locomotor activity in the fluoxetine group over time. Hyperlocomotion could relate to other research showing adolescent and adult hyperactivity following developmental fluoxetine exposure, which authors posit reflects serotonergic excitation/inhibition imbalance in cortical and striatal circuits (Houwing et al. 2019; Rodriguez-Porcel et al. 2011). This again highlights potential unintended consequences of directly interfering in the serotonergic system during sensitive neurodevelopmental windows.

Counter to original hypotheses, ketamine did not outperform fluoxetine across all domains. While demonstrating equal effectiveness for anxiety and moderate improvements in anhedonia, fluoxetine elicited more robust antidepressant-like responses. However, it remains concerning that despite alleviating offspring behaviour, fluoxetine still crosses the placenta and acts directly on the foetal brain during vulnerable developmental periods with unpredictable echo effects that may not manifest until adolescence or adulthood (Houwing et al. 2019). Ketamine, in contrast, has not been shown to accumulate in foetal tissue or breastmilk, conferring an advantage (Deng et al. 2022).

Optimal dose and timing of exposure represent other critical considerations moving forward. Our ketamine dosage and injection schedule were selected based on common antidepressant administration. But the lacklustre forced swim test results raise questions about whether this regimen sufficiently penetrates the foetal compartments to induce sustained behavioural changes. Recent work suggests the S(+)-ketamine enantiomer confers greater potency and longer-lasting upregulation of trophic factors that
may bolster neuroplasticity required for depression remission (Zhang et al. 2014; Song and Zhu 2021). Earlier initiation of treatment prior to implementing maternal stressors could also reinforce neuroprotective pathways.

Furthermore, we only examined a subset of behaviours in adult male offspring. Testing a wider range of phenotypes, including social, cognitive, sensorimotor, and self-administration assays could reveal additional domains where ketamine and fluoxetine diverge. Female offspring may also respond differently based on sex differences in placental functioning, epigenetic profiles, and neurotransmitter systems modulated by hormones (Bale et al. 2010; Migliore et al. 2021). Our modest sample sizes likewise limit generalizability and warrant studies with larger cohorts reflecting a spectrum of genetic and environmental variability to better translate findings to heterogeneous human populations.

Ketamine holds advantages over conventional antidepressants like fluoxetine due to the risks SSRIs pose to the developing foetal brain when crossing the placenta. However, timing, dosage, and interactions with the maternal-foetal interface could influence ketamine's effects as well. While maternal treatment cannot replicate direct exposure, some transmission to offspring likely still occurs during pregnancy and lactation through indirect pathways.

For example, blocking NMDA receptors impairs negative feedback on CRH neurons, increasing hypothalamic CRH which then circulates to the placenta (Sandman et al. 2011). Placental CRH secretion also inhibits active transport of maternal nutrients like zinc and fatty acids to the fetus which play vital roles in neurodevelopment (Kassotaki et al. 2021). Ketamine's antidepressant actions stem from ultimate downstream impacts on BDNF and synaptogenesis, so even minor reflective changes in levels reaching the foetus could exert actions on neuronal growth and connectivity imprinting lifelong consequences. Multi-generational studies tracking transdiagnostic outcomes are imperative.

Our future directions include testing direct foetal and early postnatal ketamine administration to model reported clinical IV administration protocols for mothers. Comparing long-term safety and efficacy to maternal exposure alone will better isolate mechanisms of transmission versus protection. Testing more chronic low-dose paradigms may provide insight on whether sustaining neurotropic factor upregulation prevents maladaptive plasticity associated with early adversity. Rational polytherapy combining ketamine with omega-3s could synergize these neuroprotective and anti-inflammatory pathways as well.

CONCLUSION

Maternal depression, anxiety, and stress during the perinatal period activate interconnected biological cascades which impair neurodevelopment and predispose offspring to psychopathology, with effects empirically shown to persist into adulthood. Typical antidepressant medications risk unintended consequences by directly disrupting neurotransmitter systems involved in brain maturation processes. Our findings indicate ketamine could treat aspects of maternal adversity-induced behavioural dysfunction without conferring adverse effects of selective serotonin reuptake inhibitors like fluoxetine. While fluoxetine elicited more powerful effects alleviating despair, equal amelioration of anxiety and anhedonia
by ketamine highlights its therapeutic promise for breaking intergenerational cycles of psychopathology. Further elucidating transmission pathways and optimizing exposure timing will illuminate best practices ensuring healthy maternal treatment and foetal development.

Declarations

Ethical Approval: The study has been registered and approved by Committee of Medical Research Ethics by College of Medicine/University of Mosul (Approval Letter CCMRE-23-20 on 13 Dec 2023)

Funding: Self-Funded

Availability of data and materials: The dataset available per requests.

Author Contribution

ZAA: study conception and design; TBT & ZAA: data collection, analysis and interpretation of results, and TBT & ZAA: manuscript preparation.

References


Figure 1

The effect of maternal stress exposure on male offspring. This figure shows the effect on both fluoxetine and ketamine used to decrease maternal stress induced depression of male offspring locomotor activity.
Figure 2
The effect of maternal stress of male offspring performance in elevated plus maze. Ketamine and fluoxetine both ameliorate anxiety-like effect.

![Bar chart showing the effect of maternal stress on sucrose fluid intake.](chart)

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Figure 3
The effect of ketamine and fluoxetine on maternal adversity induce depression-like behaviour on male offspring.

![Bar chart showing the effect of maternal stress on forced swimming test.](chart)

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Figure 4
Time of immobility in forced swimming test. Maternal stress increases offspring's immobility time in FST when compared to the mice which their dam didn't expose to such stress. Fluoxetine but not ketamine
reduces the time of immobility.