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The MAM rodent model of schizophrenia

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Abstract

Rodent models of human disease are essential to obtain a better understanding of disease pathology, the mechanism of action underlying conventional treatments, as well as for the generation of novel therapeutic approaches. There are a number of rodent models of schizophrenia based on either genetic manipulations, acute or sub-chronic drug administration, or developmental disturbances. The prenatal methylazoxymethanol acetate (MAM) rodent model is a developmental disruption model gaining increased attention because it displays a number of histological, neurophysiological and behavioral deficits analogous to those observed in schizophrenia patients. This unit describes the procedures required to safely induce the MAM phenotype in rats. In addition, we describe a simple behavioral procedure, amphetamine-induced hyper-locomotion, which can be utilized to verify the MAM phenotype.

Keywords

Methylazoxymethanol Acetate; rodent model; schizophrenia; amphetamine; locomotor activity

INTRODUCTION

Preclinical models of schizophrenia are required to gain a better understanding of disease pathophysiology and the generation of new antipsychotic medications. One model which demonstrates alterations consistent with those observed in human schizophrenia patients is the methylazoxymethanol acetate (MAM) rodent model. It has been demonstrated that the gestational (day 17) administration of MAM recapitulates a developmental disruption leading to histological (Moore et al., 2006), neurophysiological (Lavin et al., 2005; Lodge et al., 2009; Lodge and Grace, 2007), and behavioral (Lodge et al., 2009; Lodge and Grace, 2007; Moore et al., 2006) deficits analogous to schizophrenia in humans (for review see: (Lodge and Grace, 2009). In addition, this model has been used to better understand disease pathology (Lodge and Grace, 2011), the mechanisms involved in current antipsychotic treatments (Perez and Lodge, 2012; Valenti et al., 2011) and has provided a model to examine potential novel therapeutic approaches (Gill et al., 2011; Perez et al., 2012). Here we describe the procedures required to safely generate the MAM phenotype and, furthermore, provide a behavioral approach (amphetamine-induced hyper-locomotion) that can be utilized to validate this model.

BASIC PROTOCOL 1: GENERATION OF MAM PHENOTYPE

The generation of the MAM phenotype is a relatively simple process, requiring a routine intraperitoneal injection; however, the process is complicated by the hazardous nature of MAM. Here we describe the procedure in sufficient detail to minimize exposure hazard and induce reproducible deficits in histopathology, neurophysiology and behavior.

NOTE: All protocols using live animals must first be reviewed and approved by the appropriate Institutional Animal Care and Use Committee (IACUC) and conform to governmental regulations regarding the care and use of laboratory animals.

NOTE: Methylazoxymethanol acetate is a toxic and carcinogenic compound and approval for its use should be obtained from the Institutional Environmental Health and Safety Committee. In addition, the use of this compound in an animal facility should be coordinated with appropriate Laboratory Animal Resources personnel to limit potential exposure.

Materials

Rats: Female, pregnant Sprague-Dawley rats - timed to be GD17 on a Friday (Vendor: Harlan)

Standard housing cages for rats

Housing room separated from colony with limited access.

Laboratory grade balance

Methylazoxymethanol Acetate (MRI Global Chemical Carcinogen Repository: Catalog # 213)

Sterile Saline

1 ml luer-lock syringes

25G needle

Biohazard sharps container

biohazard Bags

Personal Protective Equipment including – Latex gloves, Nitrile gloves, Lab coat, Eye Protection, and Mask

Bleach; 10% v/v in water

Cavicide disinfectant

Standard rat chow softened with tap water

Protocol steps

1. Rats should be ordered such that they are timed pregnant, gestational day 17 on a Friday. As MAM is a potent carcinogen, this ensures that the high period of exposure hazard occurs over a weekend where there is lower traffic through the animal facility.
2. If possible, timed pregnant female rats should be housed in a room/cubicle isolated from the general rodent population to limit exposure hazard to other investigations and animal care staff.
3. Methylazoxymethanol acetate is a liquid at room temperature with a density of 1.17g/ml and can be stored, protected from light, at -20°C .
4. All handling of MAM should be confined to a chemical fume hood lined with clean bench covers. Personal protective equipment including double gloves (latex over nitrile) lab coat (disposable Tyvek-style clothing can be used in lieu of a standard lab coat), eye protection, and mask are essential.

5. In an eppendorf tube, MAM should be diluted with sterile saline to a final concentration of 25mg/ml by adding 20 μ l of MAM to 936 μ l of saline. Aliquots of dilute (25mg/ml) MAM can be stored at -20°C until use.
6. All gloves, pipette tips, bench covers, as well as, the empty MAM stock vial should be placed in an appropriate biohazard bag for disposal by the institutional environmental health and safety office.
7. The diluted MAM solution should be placed in a secondary container for transport to the animal facility. Prior to administration of MAM, warning labels containing chemical and emergency information should be posted on both the rodent cages and the entrance to the animal room. Include the material safety data sheet (MSDS) and the dates of high-exposure hazard, three days following administration. Coordinate with LAR personnel to ensure that staff are aware of the potential health risks. It is prudent for the PI's staff to take care of the rats for the period of high exposure hazard (three days).
8. Injections of MAM should occur late in the day (after 3pm) on gestational day 17. Personnel performing or assisting with MAM administration should don protective equipment as detailed above. Typically 4–6 pregnant rats are treated at a time with 50% receiving injections of saline and the others receiving MAM. MAM (25 mg/kg i.p.) or saline (1ml/kg i.p.) should be backfilled into a luer-lock syringe and administered into the peritoneum. Syringes should be immediately placed into a biohazard sharps container for disposal by the Institutional Environmental Health and Safety Office.

It should be noted that administration of MAM earlier than GD 17 will produce a dramatically different phenotype that does not adequately model that observed in schizophrenia patients. For example, MAM administration on GD 15 produces marked microcephaly and cortical dysplasias (Moore et al., 2006).

9. Cage cards should be labeled with treatment (i.e. MAM or Saline) and micro-isolator lids should be placed on the cages. Water-softened rat chow (mash) can be placed at the bottom of each cage.
10. PI's staff should check the rats daily over the weekend and replace food, water, and softened chow as warranted. It is essential to wear appropriate protective equipment (detailed above) when checking on the animals and replacing softened chow.
11. The cages should be changed by PIs staff on the Monday morning following MAM administration. Specifically, personal protective equipment should be worn and each pregnant dam should be transferred to a clean housing cage with fresh sawdust bedding. The contaminated bedding should be collected in biohazard bags and disposed of by the Institutional Environmental Health and Safety Office. The empty cages should be sprayed with bleach (10% v/v) and wiped clean with paper towel. Cages are further treated with Cavicide and wiped clean. Paper towel should be disposed alongside the bedding in the appropriate biohazard bag. Cages should then be given to LAR staff for further cleaning.
12. At this point, MAM-treated rats are no longer considered hazardous and can be housed with the general population under standard conditions.
13. The typical gestational period of a rat is 22 days (although this can vary) and, as such, pregnant female rats will typically give birth between Monday-Wednesday

the week following MAM or saline administration. Rats should be monitored daily to determine date of birth.

14. Male offspring should be weaned on postnatal day 21 and female rats, including the mother, should be euthanized by IACUC approved methods.
15. Multiple litters of MAM- and saline- treated rats are typically examined as adults (>8weeks of age).

BASIC PROTOCOL 2: AMPHETAMINE-INDUCED HYPER-LOCOMOTION

An augmented response to psychomotor stimulants is consistently observed in schizophrenia patients (Lieberman et al., 1987), as well as, rodent models (Flagstad et al., 2004; Gill et al., 2011; Lodge and Grace, 2007; Moore et al., 2006; Perez et al., 2012). A consistent validation of the MAM model described above is the enhanced locomotor response to a relatively low dose of the psychomotor stimulant, amphetamine. Here we describe a simple behavioral task that can be used to confirm the MAM phenotype and that we have used as a correlate for changes in dopamine system function (Gill et al., 2011; Lodge and Grace, 2007; Perez et al., 2012). It should be noted that the enhanced sensitivity to low-dose amphetamine administration is only seen following puberty in MAM-treated rats (Moore et al., 2006), although augmented responses to higher doses may be present in pre-adolescent rats (unpublished observations).

NOTE: All protocols using live animals must first be reviewed and approved by the appropriate Institutional Animal Care and Use Committee (IACUC) and conform to governmental regulations regarding the care and use of laboratory animals.

NOTE: Amphetamine is a controlled substance requiring a Schedule II license from the U.S. Drug Enforcement Administration, or equivalent, as well as local authorities. The use of amphetamine requires appropriate handling and record keeping to maintain compliance with federal, state and local laws.

Materials

Rats: Adult rats treated prenatally (GD 17) with either MAM or saline. 10–12 rats per group is typically required.

D-Amphetamine sulfate (Sigma - Aldrich)

Med Associates Rodent locomotor chamber (or equivalent)

1 ml luer-lock syringes

25G needle

Biohazard sharps container

Personal Protective Equipment including – Latex gloves & Lab coat

Laboratory grade balance

Protocol steps

1. All experiments following prenatal MAM or saline administration should be performed on rats spanning a number of different litters.
2. Adult rats should be used as differences in locomotor activity to low dose amphetamine are typically only observed following puberty in MAM-treated rats.

3. Rats treated prenatally with MAM or saline should be weighed and transported to the behavioral room where they are left to acclimate to the facility for a minimum of one hour. It is advisable for the room to be vacated for the duration of the behavioral experiment to minimize any potential confounds (i.e. freezing due to loud noises or sudden movement).
4. Following the acclimation period, rats are individually placed into a locomotor chamber where horizontal activity is monitored by beam breaks. Rats are permitted to explore for a period of 45 mins to examine baseline locomotor activity in the novel arena.

MAM-treated rats may have an increased baseline locomotor activity (see (Lodge and Grace, 2007; Perez et al., 2012)); however, baselines typically converge by 45 mins.

5. During baseline recording, amphetamine is weighed and dissolved in saline at a concentration of 0.5mg/ml.

Amphetamine is a Scheduled (SII) drug and, as such, both a federal DEA license as well as state licenses are required. Amphetamine should be kept in a locked cabinet and should only be dispensed by those lab members listed on the DEA application. Exact quantities of amphetamine should be logged with a signature as required to maintain compliance with the record keeping responsibilities of the investigator.

6. Immediately following baseline locomotor activity, all rats are administered amphetamine (0.5mg/kg, i.p.) and placed back into the locomotor arena where locomotor activity is recoded for an additional 45 mins.
7. We have recently added a second dose of amphetamine to our locomotor studies to provide a more complete examination of amphetamine-induced hyper-locomotion. Thus, a second aliquot of amphetamine is dissolved to a concentration of 2.0mg/ml.
8. After the locomotor response to 0.5mg/kg has been recorded, rats are immediately injected with a higher dose of amphetamine (2.0mg/kg i.p.) and locomotor activity is examined for a final 45 mins.
9. Data are analyzed by distance travelled and typically collapsed into 5 minute bins. Using this approach, MAM-treated rats consistently display an augmented locomotor response to the lower, 0.5mg/kg, dose of amphetamine.

COMMENTARY

Background Information

The gestational (day 17) administration of methylazoxymethanol acetate (MAM) recapitulates a developmental disruption leading to histological, neurophysiological and behavioral deficits analogous to schizophrenia in humans (for review see: (Lodge and Grace, 2009). Specifically, MAM-treated rats display subtle histopathologies including decreases in cortical thickness with corresponding increases in neuronal density (Moore et al., 2006) and decreases in parvalbumin expression (Lodge et al., 2009; Penschuck et al., 2006). While mirroring deficits in histopathology are relatively straight forward, it is impossible to directly measure symptoms of schizophrenia e.g., hallucination, delusion and thought disorder) in rodents. Thus, MAM-treated rats have been examined in variety of behavioral paradigms that are considered to be valid translational models due to similar measures of assessment in rats and humans. Specifically, deficits have been observed in sensorimotor gating (Le Pen et al., 2006; Moore et al., 2006; Talamini et al., 2000), latent inhibition (Lodge et al., 2009), cognitive function (Gastambide et al., 2011; Le Pen et al., 2006; Perez

et al., 2012) and hyper-responsivity to psychomotor stimulants (Flagstad et al., 2004; Gill et al., 2011; Lodge and Grace, 2007; Moore et al., 2006; Perez et al., 2012). Furthermore, aberrant dopamine and hippocampal system function, consistently observed by imaging studies in patients (Abi-Dargham et al., 2009; Schobel et al., 2009), have been demonstrated by electrophysiological studies in MAM-treated rats (Lodge and Grace, 2011). Thus, prenatal MAM administration produces a developmental disruption leading to schizophrenia-like alterations, thus providing a unique model to examine pathophysiology and potential novel therapeutic advances. Indeed, this rodent model has been recently utilized to better understand mechanisms underlying current antipsychotic drug action (Perez and Lodge, 2012; Valenti et al., 2011) and has enabled the identification of potential novel pharmacological (Gill et al., 2011) and non-pharmacological (Perez et al., 2012) therapeutics for the treatment of schizophrenia.

Critical Parameters & Troubleshooting

Pregnant Females—It is imperative that the day of gestation, typically confirmed by the presence of a copulatory plug, be accurately determined. The optimal timing for MAM administration (in the context of schizophrenia) was determined to be GD 17 (Moore et al., 2006), as at this time neuronal proliferation has peaked in many cortical regions and is essentially complete in subcortical regions (Bayer and Altman, 1995). Indeed, consistent with the brain regions known to be altered in schizophrenia patients, MAM administration on GD 17 produces relatively selective effects on paralimbic frontal and temporal cortices (Moore et al., 2006).

Methylazoxymethanol Acetate—It should be remembered that MAM is a potent toxin and carcinogen (Ganote and Rosenthal, 1968; Smith, 1966; Zedeck et al., 1970). Thus, it is essential that all precautions detailed above be followed in order to decrease the risk of exposure hazard, not only to the people performing the MAM injections, but to also limit exposure to other investigators and animal care staff.

Amphetamine-Induced Hyper-locomotion—The augmented response to low-dose amphetamine is a consistent observation in MAM-treated rats (Flagstad et al., 2004; Gill et al., 2011; Lodge and Grace, 2007; Moore et al., 2006; Perez et al., 2012). It should be noted, however, that MAM-treated rats have been demonstrated to display an enhanced baseline locomotor response that may confound the interpretation of the amphetamine data. Thus, it is recommended that baseline activity be monitored for at least 45 mins in order for any observed differences in baseline distance travelled to reach convergence.

Anticipated Results

Following the above protocols should produce litters of approximately 8–12 MAM- or saline- treated offspring that can be used for molecular, anatomical, neurophysiological, or behavioral studies. We have included an additional protocol, amphetamine-induced hyper-locomotion, that is consistently reported to be augmented in MAM-treated rats when compared to prenatal saline-treated rats. It is anticipated that MAM-treated rats will display a statistically significant, enhanced locomotor response to the low (0.5mg/kg i.p.) dose of amphetamine. This observation is consistent with the enhanced response to psychostimulant administration observed in schizophrenia patients (Lieberman et al., 1987).

Time Considerations

The generation of MAM- and saline- treated rats typically requires a number of months so it is important to plan well in advance. We purchase timed-pregnant female rats that are treated with MAM or saline on the Friday following arrival. Animal care takes three days

(high exposure hazard) and offspring are born approximately 4 days following administration. Weaning takes place three weeks later, on postnatal day 21, and rats are routinely housed until adulthood (>8weeks).

Following 1 hour acclimatization to the room, amphetamine-induced locomotion studies take 2.25 hours per rat to complete. However, multiple animals can be run at the same time in separate locomotor chambers. We typically run 4 rats at a time with a throughput of 16 – 20 rats per day.

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