Schizophrenia Research xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Schizophrenia Research

journal homepage: www.elsevier.com/locate/schres

Impaired contextual fear-conditioning in MAM rodent model of schizophrenia☆

Kathryn M. Gill *, Sarah A. Miller, Anthony A. Grace

University of Pittsburgh, Pittsburgh, PA 15260, Departments of Neuroscience, Psychiatry and Psychology, USA

ARTICLE INFO

Article history:
Received 16 May 2017
Received in revised form 31 August 2017
Accepted 31 August 2017
Available online xxxx

Keywords:
Schizophrenia
Animal models
Fear learning
Dopamine
Context discrimination
Electrophysiology

ABSTRACT

The methylazoxymethanol acetate (MAM) rodent neurodevelopmental model of schizophrenia exhibits aberrant dopamine system activation attributed to hippocampal dysfunction. Context discrimination is a component of numerous behavioral and cognitive functions and relies on intact hippocampal processing. The present study explored context processing behaviors, along with dopamine system activation, during fear learning in the MAM model.

Male offspring of dams treated with MAM (20 mg/kg, i.p.) or saline on gestational day 17 were used for electrophysiological and behavioral experiments. Animals were tested on the immediate shock fear conditioning paradigm, with either different pre-conditioning contexts or varying amounts of context pre-exposure (0–10 sessions). Amphetamine-induced locomotor activity and dopamine neural activity was measured 1-week after fear conditioning.

Saline, but not MAM animals, demonstrated enhanced fear responses following a single context pre-exposure in the conditioning context. One week following fear learning, saline rats with 2 or 7 min of context pre-exposure prior to fear conditioning also demonstrated enhanced amphetamine-induced locomotor response relative to MAM animals. Dopamine neuron recordings showed fear learning-induced reductions in spontaneous dopamine neural activity in MAM rats that was further reduced by amphetamine. Apomorphine administration confirmed that reductions in dopamine neuron activity in MAM animals resulted from over excitation, or depolarization block.

These data show a behavioral insensitivity to contextual stimuli in MAM rats that coincide with a less dynamic dopamine response after fear learning.

1. Introduction

The hippocampus plays a pivotal role in context discrimination functions, including those necessary for fear learning (Frankland et al., 1998; McDonald et al., 2004) (Frohardt et al., 1999; Holt and Maren, 1999; Quintero et al., 2011; Young et al., 1995). Schizophrenia is a complex psychiatric disorder with known hippocampal dysfunction and context discrimination deficits (Benes, 2015; Guillaume et al., 2015; MacDonald et al., 2005; MacDonald et al., 2003; Schobel et al., 2013; Schobel et al., 2009a; Schobel et al., 2009b; Servan-Schreiber et al., 1996; Siever et al., 2002; Talamini and Meeter, 2009), such as inappropriate memory generalization or an inability to ignore irrelevant stimuli (Gal et al., 2005; Ivleva et al., 2012; Jazbec et al., 2007; Racsmany et al., 2008; Roiser et al., 2009; Shohamy et al., 2010; Warren and Haslam, 2007). Patients are also unable to modulate hippocampal activation during recognition memory, especially in response to novel stimuli (Ivleva et al., 2012; Schott et al., 2015). The hippocampus shows aberrant increases in activity preceding transition to psychosis, and there is a proposed link between hippocampal activation, morphological changes, and severity of positive symptoms (e.g. hallucinations and delusions) (Arnold et al., 2015; Jensen et al., 2008; Narr et al., 2004; Schobel et al., 2009a; Talati et al., 2014; Zierhut et al., 2013).

Abnormal hippocampal activity in schizophrenia likely underlies the pathological alteration of the dopamine system (Abi-Dargham et al., 1998; Abi-Dargham et al., 2004; Abi-Dargham et al., 2009; Breier et al., 1997; Howes et al., 2013; Laruelle and Abi-Dargham, 1999). The methylazoxymethanol acetate (MAM) rodent neurodevelopmental model of schizophrenia has demonstrated that increased dopamine activity measured both electrophysiologically and behaviorally can be attributed to disrupted GABA-mediated inhibition within the ventral

☆ This work was supported by United States Public Health Service Grants MH57440 (A.A.G.) and MH105782 (K.M.G.)

* Corresponding author at: University of Pittsburgh, Department of Neuroscience, A210 Langley Hall, Pittsburgh, PA 15260, USA.
E-mail address: gillkm@pitt.edu (K.M. Gill).

http://dx.doi.org/10.1016/j.schres.2017.08.064
0920-9964/

hippocampus (Gill and Grace, 2014; Gill et al., 2011; Lodge et al., 2009; Lodge and Grace, 2007). How this hyperactivity of the dopamine system relates to potential context processing deficits is not clear, although there is evidence from patients of an altered dopamine activation in response to contextual novelty (Heinz and Schlagenhauf, 2010). In normal rats, increased dopamine release in limbic brain regions is associated with contextual fear learning (Martinez et al., 2008). Elevated dopamine activation in MAM rats resulting from hippocampal overdrive could obscure fear learning related changes in dopamine release.

We examined context processing deficits in MAM rats using the immediate shock fear conditioning paradigm. This task requires the rapid and accurate retrieval of contextual information acquired during a pre-exposure session (Huff et al., 2006; Matus-Amat et al., 2007; Robinson-Drummer and Stanton, 2015; Rudy et al., 2002). Accurate fear learning in this paradigm requires the hippocampus, a region with known perturbation in the MAM model (Lodge et al., 2009; Lodge and Grace, 2007).

The ability to discriminate between two distinct contexts following fear conditioning, as well as the impact of repeated presentation of contextual stimuli on performance, was assessed in the MAM model. It was anticipated that MAM rats would require more extensive context pre-exposure to produce a similar reduction of fear responses that is observed in normal rats. In contrast, more extensive context exposure may instead be necessary for increasing fear responses in MAM rats to comparable levels accomplished with less context exposure in normal rats due to a malfunctioning ventral hippocampus. Typically, repeated presentation of a discrete stimulus prior to conditioning lessens its associative strength in a process described as latent inhibition. Deficits in latent inhibition in schizophrenia are inconsistent and appear dependent on medication status or disease duration (Gal et al., 2009; Lubow et al., 2000; Rascle et al., 2001; Swerdlow et al., 1996; Vaitl et al., 2002; Williams et al., 1998). However, there is compelling evidence from animal models that latent inhibition results in both increased dopamine release in the nucleus accumbens and requires intact processing in the ventral hippocampus, especially via the primary output of the ventral subiculum (Gray et al., 1995; Peterschmitt et al., 2005). Electrophysiological recordings from dopamine neurons in the ventral tegmental area are an indirect measure of underlying hippocampal hyperactivity in the MAM model (Lodge et al., 2009; Lodge and Grace, 2007) and other constructs (stress (Valenti et al., 2012), pilocarpine model of temporal lobe epilepsy (Cifelli and Grace, 2012), amphetamine (Lodge and Grace, 2008)). Consequently, whether there was a persistent consequence of contextual fear learning on the dopamine system of MAM animals was measured via electrophysiological recordings from the ventral tegmental area or the locomotor response to amphetamine 7–10 days after fear conditioning.

2. Methods

Experiments were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee at the University of Pittsburgh. Animals were housed in a temperature (22 °C) and humidity (47%) controlled environment with a 12-hour light/dark cycle (lights on 7 a.m.) with ad libitum access to both food and water. For behavioral experiments, animals were housed in a reverse light cycle room (lights on 7 p.m.) and tested during the lights-off cycle. Behavioral experiments began 7 days after animals were placed in the reverse light cycle room.

2.1. Methylazoxymethanol treatment

Timed pregnant female Sprague Dawley rats (Envigo) were obtained on gestational day (GD) 14. MAM (20 mg/kg, i.p.) or saline (1 ml/kg, i.p.) was administered on GD 17, as described previously (Gill et al., 2011; Lodge et al., 2009; Lodge and Grace, 2007; Moore et al., 2006). Male pups were weaned (day 21) and pair-housed with littermates until use in electrophysiological or behavioral experiments (approximately 3–4 months). Each MAM and Saline litter varied in the overall number of male offspring produced (range:3–7). However, animals from individual MAM and Saline litters were counterbalanced across the fear conditioning treatment groups to avoid a potential litter effect. Therefore within any given behavior or electrophysiological group (e.g. fear conditioned MAM rats with 1 pre-exposure), rats from different litters were represented. In addition, the control MAM and saline animals used for behavioral and electrophysiological comparisons were offspring of MAM- and saline-treated dams that did not undergo any fear conditioning but were exposed to the testing environment.

2.2. Exps. 1 and 5: context pre-exposure during fear conditioning

All animals were handled for a minimum of 2 days (2 min/day) prior to context pre-exposure and training in the immediate shock fear conditioning paradigm (Barrientos et al., 2002; Huff and Rudy, 2004; Rudy et al., 2002). Experiments varied (details below) by the type of context pre-exposure (Exp.1) or the number of context pre-exposures (Exp.2). Animals were randomly assigned to the experimental conditions and counterbalanced (Fig. 1).

2.2.1. Exp.1

Context A and Context B varied along several dimensions (Fig. 1C). On Day 1, animals explored one of two conditioning contexts (Fig. 1A) for 10 min. 24 h after pre-exposure, all animals were placed in Context A for 2 min, terminating in a 2-s, 0.5 mA shock through the grid floor. Animals were immediately returned to the home cage. 24-hours after conditioning, animals were placed in **Context A** and freezing behavior was measured (5 min). Subsequently, 24-hours after the Context A test, animals were placed in **Context B** and freezing behavior was measured (5 min).

2.2.2. Exp.2: Amphetamine-induced locomotor activity post-fear conditioning

7–10 days following fear conditioning (Exp.2), animals received acute injections of D-Amphetamine hemisulfate salt (Sigma; 0.5 mg/kg, i.p.). This dose typically produces a greater locomotor response in MAM animals relative to saline controls (Gill et al., 2014; Gill et al., 2011; Lodge et al., 2009). Baseline (30 min) and post-amphetamine (90 min) locomotor activity was measured by beam breaks in the x–y plane of an open field arena (Coulbourn Instruments, TruScan software, Allentown, PA). Total distance travelled (cm) was computed (5 min epochs).

2.2.3. Exps. 3 and 4: DA neuron electrophysiological recordings post-fear conditioning

7–10 days following fear conditioning (Exp.2), single-unit electrophysiological recordings were conducted from the ventral tegmental area (VTA) of animals anesthetized with chloral hydrate (See Supplemental methods).

Some animals received a dose of amphetamine (0.5 mg/kg, i.p.) 30 min prior to dopamine recordings. In another subset of animals, the VTA was sampled in both right and left hemispheres pre- and post-apomorphine (Sigma; 20 μg/kg, i.v.) administration, respectively. Since D2 auto-receptors are more responsive to dopamine than the post-synaptic receptors, low doses of apomorphine (range of doses applied 20–120 μg/kg, i.v.) can preferentially stimulate D2 autoreceptors and inhibit dopamine neuron firing (Akaoka et al., 1992; Bunney and Grace, 1978; Chiodo et al., 1984; Grace and Bunney, 1985; Valenti et al., 2011). The doses of apomorphine used in the present study are consistent with the auto-receptor selectivity.

30 min after apomorphine administration, the contralateral VTA was sampled in an identical manner. (See Supplement for histological methods.)

Please cite this article as: Gill, K.M., et al., Impaired contextual fear-conditioning in MAM rodent model of schizophrenia, Schizophr. Res. (2017), http://dx.doi.org/10.1016/j.schres.2017.08.064