Neurobiology of Learning and Memory 92 (2009) 89–98

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

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

Intrahippocampal administration of an NMDA-receptor antagonist impairs spatial discrimination reversal learning in weanling rats

Deborah J. Watson *, Mark E. Stanton

University of Delaware, Department of Psychology, 108 Wolf Hall, Newark, DE 19716, United States

ARTICLE INFO

Article history:
Received 11 December 2008
Revised 16 February 2009
Accepted 18 February 2009
Available online 25 February 2009

Keywords:
Development
Reversal Learning
Hippocampus
MK-801
Rat
Spatial Learning

ABSTRACT

Systemic administration of MK-801, an NMDA-receptor antagonist, impairs reversal learning in weanling rats [Chadman, K.K., Watson, D.J., & Stanton, M.E. (2006). NMDA-receptor antagonism impairs reversal learning in developing rats. *Behavioral Neuroscience*, *120*(5), 1071–1083]. The brain systems responsible for this effect are not known in either adult or young animals. This study tested the hypothesis that hippocampal NMDA receptors are engaged in weanling-age rats during spatial discrimination reversal training in a T-maze. In Experiment 1, 26-day-old Long-Evans rats (P26) showed a dose-related impairment on this task following bilateral intrahippocampal administration of either 2.5 or 5.0 μg MK-801 or saline vehicle during the reversal training phase only. In Experiment 2, P26 rats were trained on the same task, but received intrahippocampal MK-801 (2.5 μg) during acquisition, reversal, both, or neither. MK-801 failed to impair acquisition, ruling out nonspecific "performance effects" of the drug. MK-801 impaired reversal irrespective of drug treatment during acquisition. NMDA-receptor antagonism in the hippocampus is sufficient to account for the previously reported effects of systemic MK-801 on reversal of T-maze position discrimination.

The N-methyl-D-Aspartate (NMDA) receptor subtype of glutamate receptors plays a substantial role in neural physiology, synaptic plasticity, and behavioral learning and memory. These roles include, but are not limited to, the molecular/cellular basis of short- and long-term memory formation, the induction and maintenance of long-term potentiation (LTP), as well spatial learning and memory that depends upon the hippocampus (Morris, Anderson, Lynch, & Baudry, 1986; O'Keefe & Nadel, 1978; Shapiro, 2001).

NMDA receptors are heavily concentrated in the hippocampal formation, cortex, and striatum (Wong et al., 1986; Wong, Knight, & Woodruff, 1988). These same brain regions are essential for spatial learning and memory, contextual memory, and higher-order cognitive learning tasks in adult animals. It is currently unknown if these same brain regions are involved in reversal learning during early ontogeny.

The NMDA-receptor subunit representation is not the same across the lifespan. The mRNA transcripts of all NMDA-receptor subunits peak by post-natal day (P) 20 (except NR2D, which peaks at P7, and then decreases to adult levels) (Monyer, Burnashev, Laurie, Sakmann, & Seeburg, 1994). The most apparent changes occur by the end of the 3rd post-natal week of life (P21). The NR2B and NR2D subunits have mRNA expression that is first detected during prenatal development across most brain regions at embryonic day (E) 14 and then expression declines in adulthood. NR2A and NR2C subunits are barely detectable at birth, and mRNA transcripts are first expressed post-natally (approximately P7) and increase into adulthood, with NR2A expression highest in forebrain regions and NR2C particularly in the cerebellum (Monyer et al., 1994). A similar pattern of expression of NMDA-receptor subunits is seen across mouse development (Vallano, 1998). In general, the contribution of NR2B decreases across development, while NR2A has an increased contribution to NMDA-receptor function (Cull-Candy, Brickley, & Farrant, 2001). In addition to these changes in receptor subunit expression across development, expression of LTP in CA1 pyramidal cells also changes with maturation; such that LTP first occurs by P5 and reaches maximal response around P15 (Harris & Teyler, 1984; Teyler, Perkins, & Harris, 1989). Hippocampal dendritic spine formation follows a similar timeline in developing rats (Bourne & Harris, 2008; Harris, 1999). In contrast with these neurobiological studies, much less is known about the role NMDA receptors play in the ontogeny of reversal learning and the neural substrates underlying this form of learning.

Rats as young as P7-15 can acquire and reverse a position habit using suckling as a reward (Green & Stanton, 1989; Kenny & Blass, 1977). Reversal learning of a discrimination is cognitively more demanding than initial acquisition of a discrimination (Dias, Robbins, & Roberts, 1997). Reversal learning is dependent upon three behavioral processes: (1) memory of the initially acquired response, (2) learned suppression of this initially acquired

* Corresponding author. Fax: +1 302 831 3645.
E-mail address: dwatson@psych.udel.edu (D.J. Watson).

1074-7427/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.nlm.2009.02.005

response, (3) learning the new (competing) response. A small number of studies have investigated the role of NMDA in learning in developing animals (Chadman, Watson, & Stanton, 2006; Griesbach & Amsel, 1998; Griesbach, Hu, & Amsel, 1998; Highfield, Nixon, & Amsel, 1996). NMDA-receptor involvement was confirmed in both spatial and olfactory reversal learning and nonspatial working memory in weanling rats. Previous work in our lab has demonstrated that NMDA-receptor antagonism with MK-801 impaired spatial reversal performance in P21-30 rats (Chadman et al., 2006). The role different brain regions play in this reversal learning deficit in young rats is currently unknown.

Two experiments examined the effect of NMDA-receptor antagonism within the hippocampus on spatial discrimination reversal learning in weanling rats. Experiment 1 evaluated different doses of MK-801 to determine for the first time whether antagonism of hippocampal NMDA receptors impairs spatial reversal learning in P26 rats. Experiment 2 sought to determine if the MK-801 impairment was specific to the reversal learning phase relative to the acquisition phase by administering MK-801 before acquisition, reversal, both, or neither. Based on prior studies with systemically administered MK-801, we predicted that the highest dose of MK-801 would lead to the greatest impairment and the lowest dose of MK-801 would modestly impair reversal learning performance (Experiment 1). We also predicted that this effect would be selective for the reversal learning phase, sparing acquisition performance, eliminating the potential role of "performance" or state-dependent learning effects (Experiment 2).

1. Experiment 1

Systemically administered MK-801 selectively impairs reversal learning in weanling rats (Chadman et al., 2006). In adult rats and mice, the hippocampal system plays a role in spatial learning (Morris, 2006; Morris et al., 2003; Shapiro, 2001; Wang & Cai, 2006) and more specifically in discrimination reversal learning (Bardgett et al., 2003; Oliveira, Bueno, Pomarico, & Gugliano, 1997). However, the role of the hippocampus in reversal learning has not been examined in developing rats. Experiment 1 was designed to evaluate whether an infusion of MK-801 into the dorsal hippocampus would impair reversal learning in weanling rats, and whether the MK-801 impairment was dose-dependent.

Three treatment groups received eight blocks of spatial discrimination training (four blocks in acquisition, and four in reversal). All groups received bilateral infusions in dorsal hippocampus (0.5 μl per side). Separate groups were administered a high dose of MK-801 (5.0 μg per side); an intermediate dose (2.5 μg per side); or vehicle (sterile saline). If hippocampal receptors are involved in the effects of systemic MK-801 administration seen previously, then the present experiment should reveal dose-related impairment of reversal learning.

1.1. Method

1.1.1. Subjects

Thirty-one (16 female, 15 male) Long-Evans rat pups derived from 21 litters served as subjects. Litters were housed in the laboratory vivarium with ad libitum access to food and water on a 12:12 hr light-dark cycle (onset at 0700 hr). Litters were culled to eight pups (usually four males and four females) on P3 (date of birth is P0). A subset of the pups from the 21 litters were used for Experiment 1, the remaining pups were assigned to other ongoing studies. The pups were weaned on P21 and housed with same-sex littermates until P23. Weaned pups had uninterrupted access to food and water until the onset of behavioral procedures. The surgical procedure began on approximately P23 (range: 22–23). Subjects were housed individually in cages following surgery for the duration of the experiment. Pups were allowed to recover from surgery for 1 day before the onset of deprivation (see *Procedure* below). The average weight at deprivation for subjects was 59.8 ± 1.04 g (range: 51.0–77.0 g). ANOVA performed on the deprivation weight data did not reveal differences between treatment groups ($F < 0.61$).

One pup was discarded from analysis because it did not consume the reward following drug administration. Of the remaining 30 pups, seven pups were excluded from further analysis following histological analysis of cannula placement. These pups were excluded due to incorrect cannula placements in the corpus callosum or overlying cortex rather than the dorsal hippocampus. Data from the remaining 23 pups are reported below.

1.1.2. Surgery

Our surgical procedure for weanling rat intrahippocampal cannula implantation has been described previously (see Watson, Herbert, & Stanton, 2009). Commercially-obtained cannulas (Plastics One, Roanoke, VA) were implanted bilaterally under stereotaxic guidance in the brains of weanling rats under ketamine/xylazine anesthesia (52.2–60.9 mg/kg ketamine/7.8-9.1 mg/kg xylazine in a 0.7–0.85 ml/kg injection volume). Buprenorphine (0.03 mg/kg in a volume of 0.05 ml/100 g) was also administered subcutaneously to alleviate pain during and following the surgical procedure. The dorsal skull surface was exposed and small holes were drilled in the skull based on stereotaxic coordinates adjusted empirically from an atlas of the developing rat brain (Sherwood & Timiras, 1970). Guide cannulas were bilaterally implanted in the dHPC (AP + 2.6 mm, ML ± 2.3 mm, DV −1.8 mm). All AP and ML coordinates were based on interaural coordinates as measured from the horizontal zero plane, such that the ear bars and incisor bar were set to zero (Sherwood & Timiras, 1970). The cannula assembly was secured to the hooks implanted in the skull with dental acrylic at the end of surgery (Gilbert & Cain, 1980; Stanton & Freeman, 1994). Dummy cannulas were inserted into the guide cannula to prevent obstruction until infusions were made. Following antibiotic ophthalmic ointment application, rats were then returned to their home cages with food and water. Rats were monitored and kept warm until they recovered from anesthesia. Rats received 1 day of recovery prior to the deprivation procedure that started the T-maze protocol. This amount of recovery time has been found sufficient for weanling/juvenile rats having undergone stereotaxic surgery (Freeman, Rabinak, & Campolattaro, 2005; Watson et al., 2009).

1.1.3. Drugs

The experiment involved administration of the non-competitive NMDA-receptor antagonist, dizocilpine (MK-801). MK-801 was purchased commercially from Tocris (Ellisville, MO). It was dissolved in sterile saline. (Treatment doses are described under drug infusion procedure below.)

1.1.4. Drug infusion procedure

The drug infusion procedure was performed as described by Watson et al. (2009). Five minutes prior to the start of each position habit training session, the awake rats were bilaterally intrahippocampally infused. Vehicle infusions were made before the acquisition session to all treatment groups, and MK-801 or vehicle was administered before the reversal session. Rats were gently held while the dummy cannulas were removed and an injection cannula was lowered through each guide cannula extending 1 mm below the tip. The injection cannula was connected to polyethylene tubing attached to a 10 μL Hamilton syringe mounted on a microinfusion pump. MK-801 was dissolved in sterile saline at a concentration of either 5 or 10 μg per μL and delivered at a rate of