Neurobiology of Learning and Memory 93 (2010) 275–282

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

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

Impaired active avoidance learning in infant rats appears to be related to insufficient metabolic recruitment of the lateral septum

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ARTICLE INFO

Article history:
Received 9 September 2009
Revised 23 October 2009
Accepted 15 November 2009
Available online 18 November 2009

Keywords:
Functional imaging
Shuttle-box
Aversive learning
Negative reinforcement
Limbic system

ABSTRACT

The temporal dissociation between early information acquisition and output of complex behaviors is a common principle during development. Thus, although infant rats are not able to generate sufficient avoidance behavior during two-way active avoidance (TWA) training they obviously deposit a certain "memory trace" (Schäble, Poeggel, Braun, & Gruss, 2007). The ontogeny of learning is probably mirrored by the maturing functionality of different basal forebrain regions.

Two of the basal forebrain regions involved in TWA learning are the medial septum/diagonal band of Broca (MS/DB), which is essential for the encoding and retrieval of memory and the lateral septum (LS) that plays a role in the generation of behavior.

Mapping 2-fluoro-deoxy-glucose utilization in freely behaving animals, the aim of this study was to assess the functional recruitment of the MS/DB and LS in infant (P17–P21) and adolescent (P38–P42) rats during the first (acquisition) and fifth (retrieval) TWA training.

Metabolic activity in the MS/DB was similar in both age groups during acquisition and retrieval indicating that this region is already mature in the infant rat. In contrast, metabolic activity in the LS was generally lower in the infant rats suggesting that this region is not yet fully functional during P17 and P21. This insufficient recruitment may be one reason for the poor TWA performance of infant rats.

Finally, the LS displayed significantly higher activity during acquisition than during retrieval indicating that the highest amount of energy is consumed during the initial learning phase.

1. Introduction

It is known that young rats show a poor two-way active avoidance (TWA) performance compared to adolescent and adult rats (Bauer, 1978; Izquierdo, Salzano, Thome, & Thaddéu, 1975; Kudryashova, 2006; Schäble et al., 2007; for a general introduction into TWA behavior see Anderson (2000)).

We recently confirmed that infant rats (P17–P21) were not able to establish adequate TWA behavior during shuttle-box training. Nevertheless, as adults these animals showed improved avoidance learning (Schäble et al., 2007). Thus, although the young rats failed to generate adequate avoidance behavior during infancy, early acquisition of experience seems to leave an enduring "memory trace" which can facilitate learning in adulthood.

Two morpho-functional different basal forebrain structures, the medial septum/diagonal band of Broca (MS/DB) and the lateral septum (LS), play a central role in higher cognitive as well as emotional processes (Numan, 2000). Lesion studies revealed that the MS/DB and LS differentially contribute to the performance in TWA tasks (Gray & McNaughton, 1983; Hepler, Wenk, Cribbs, Olton, & Coyle, 1985; Numan, Ward, & Clark, 1982; Sengstock et al., 1992; Sparks & LeDoux, 2000; Torras-Garcia, Costa-Miserachs, Morgado-Bernal, & Portell-Cortés, 2003). The MS/DB primarily integrates information about the biological significance of episodes and events, i.e. contextual information. This basal forebrain region is projecting to the entire cortex including hippocampus and cortical amygdala and innervated by entorhinal afferents and noradrenergic fibers from the locus coeruleus (Alonso & Köhler, 1984; Calandreau, Jaffard, & Desmedt, 2007; Givens & Sarter, 1997; Haghdoost-Yazdi, Pasbakhsh, Vatanparast, Rajaei, & Behzadi, 2009; Lindvall & Stenevi, 1978). In contrast, the LS plays an important role in the generation of behavior enabling the individual to increase interactions with reinforcing stimuli (Sheehan, Chambers, & Russell, 2004). The LS is innervated by the hippocampus and the extended amygdala but also receives prominent dopaminergic and serotonergic afferents from the ventral mesencephalon and raphe nuclei, respectively (Haghdoost-Yazdi et al., 2009; Jakab & Leranth, 1995; Lindvall & Stenevi, 1978; Seifert et al., 1998).

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doi:10.1016/j.nlm.2009.11.001

The regulation of modulatory states interfering with learning and memory – like attention and motivation – strongly depends on the integrity of the MS/DB and LS (Bannerman, Matthews, Deacon, & Rawlins, 2004; Sheehan et al., 2004; Treit & Menard, 2000).

Thus, the aim of this study was to assess the differential recruitment of the MS/DB and LS in different phases of TWA learning and their relation to age in the intact brain. We circumvented the problem of tissue damage applying the 2-fluoro-deoxyglucose (glucose) utilization method. This non-invasive technique allows the quantitative mapping of regional brain activity in freely moving animals leaving the brain morpho-functionally intact during the entire behavioral task (Konkle & Bielajew, 2004; Sokoloff et al., 1977).

Based on the differential morpho-functional characteristics of the MS/DB and LS, we predict that (1) metabolic activity of the MS/DB does not show an age difference, as its mature function is necessary for the deposition of a memory trace which is recruited during adulthood (Schäble et al., 2007). (2) Metabolic activity of the LS is still immature in infant rats since they not display a sufficient avoidance response. (3) Metabolic activity is higher in the acquisition group than in the retrieval group in adolescent rats, as performing a learned task should require less neuronal activity.

2. Materials and methods

2.1. Animals

A total of 68 female Wistar rats (Shoe rats) from the breeding colony at the Leibniz Institute for Neurobiology Magdeburg were used for the experiments.

Female rats were used because in pilot experiments and in our recent study TWA learning performance in females was more homogenous than in males (Schäble et al., 2007). Animals were either infant (P17–P21, 9 litters) or adolescent (P38–P42, 9 litters, Table 1). All experimental protocols were approved by the ethics committee of the government of Saxony-Anhalt according to the German guidelines for the care and use of animals in laboratory research. The experiments were performed in accordance with the European Communities Council Directive (86/609/EEC). Experiments were generally carried out between 8:00 and 12:00 a.m.

2.2. Housing

At the day of birth (P0) litters were standardized to five female and five male pups per dam. The infant rats were kept with their mothers and siblings until P21. At P21 females were weaned from their mother and housed together until P42. All animals were kept in translucent standard laboratory cages Type IV (E. Becker & Co. GmbH, Germany, Castrop-Rauxel) under controlled laboratory conditions (temperature: 21 ± 2 °C; humidity: 55 ± 5%; artificial 12 h/12 h light/dark cycle) with access to food and water ad libitum. Cage cleaning was done once a week.

Table 1
Experimental groups.

	Infant rats					Adolescent rats				
Age (P)	17	18	19	20	21	38	39	40	41	42
Acquisition group	–	–	–	–	*t*[a]	–	–	–	–	*t*[a]
Novelty group	–	–	–	–	exp[a]	–	–	–	–	exp[a]
Retrieval group	*t*	*t*	*t*	*t*	*t*[a]	*t*	*t*	*t*	*t*	*t*[a]
Familiarity group	exp	exp	exp	exp	exp[a]	exp	exp	exp	exp	exp[a]

[a] Injection of 2-FDG (18 μCi /100 g b.w.); (*t*) = training, (exp) exposure to the shuttle-box without CS and UCS presentation.

2.3. Behavioral experiments

Experiments were conducted using fully automated shuttle-boxes located in ventilated and sound-protected cubicles (TSE Systems GmbH, Germany, Bad Homburg). Infant rats were trained in shuttle-boxes of 30.3 × 23.0 × 20.5 cm size (length × depth × height), equipped with a floor grid of 0.4 cm diameter bars spaced 0.9 cm apart. Adolescent rats were trained in shuttle-boxes of 48.5 × 23.0 × 20.5 cm size with a floor grid of 0.9 cm diameter bars spaced 1.8 cm apart. Shuttle-boxes were bisected by a vertical wall (non-transparent polyvinyl chloride plate), which contained a door allowing the animal to freely move to the opposite compartment. Loudspeakers were placed on top of each compartment. Infrared light beams permanently determined the position of the animal. For system control and data acquisition the shuttle exe software (TSE Systems GmbH, Germany, Bad Homburg) was used. To minimize odor cues, shuttle-boxes were cleaned with 70% ethanol after each animal.

2.4. Training paradigm and behavioral data

Each training session started with 3 min of habituation allowing the animals to freely explore the shuttle-box. Afterwards, learning trials were applied as follows (Fig. 1): the conditioned stimulus (CS), a tone of 2.4 kHz frequency and 80 dB loudness, was presented for 5 s maximally. Afterwards, the unconditioned stimulus (UCS), a 600 μA foot-shock, was added for a maximal duration of 15 s. Inter-trial interval (ITI) consisted of 40 s. The following parameters were recorded and analyzed: *number of avoidance reactions*, *avoidance latency*, *number of escape reactions*, *escape latency*, *number of failures* and the *number of inter-trial changes*.

2.5. Experimental groups

Two training groups (acquisition vs. retrieval) and two respective control condition groups (novelty vs. familiarity) were set up. Individuals of infant and adolescent litters were randomly assigned to one of the four experimental groups (Table 1) and trained/exposed as follows: (I) *Acquisition*: animals had to perform 50 learning trials at P21 ($n = 9$) or P42 ($n = 9$). (II) *Novelty*: animals were exposed to the shuttle-box without presentation of CS and UCS at P21 ($n = 9$) or P42 ($n = 8$). The time of shuttle-box exposure was kept in the same range like the time the animals in the acquisition group needed to perform the 50 trials, i.e. about 40 min. (III) *Retrieval*: animals had to perform a series of 50 trials on five consecutive days from P17 to P21 ($n = 8$) or P38 to P42 ($n = 9$). (IV) *Familiarity*: animals were exposed to the shuttle-box without CS

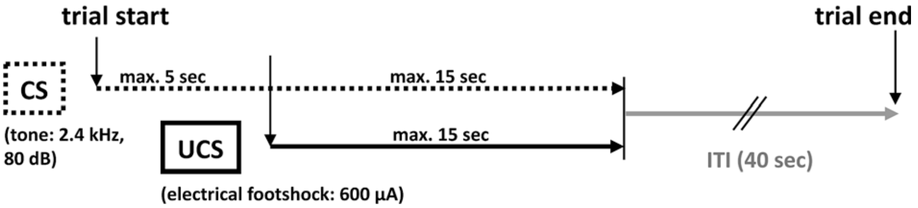

Fig. 1. Illustration of the trial paradigm. CS: conditioned stimulus, UCS: unconditioned stimulus, ITI: inter-trial interval.