The ontogeny of spatial memory in rats

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Table of Contents

Abstract

Been able to navigate through the environment is essential for every mobile species. Up to date, it is quite understood how spatial representations are formed, how these representations serve our everyday memories and what are the brain mechanisms that underlie these processes. Most of this knowledge has been acquired upon studies in adults while the development of the spatial memory capability across ontogeny remains less understood. Recent findings have shown that the brain structures that support spatial cognition, such as the hippocampus, have a protracted development including a critical period. Yet, the studies conducted to investigate the developmental trajectory of hippocampal-dependent spatial capabilities are scarce. The present thesis addresses three open questions regarding the ontogeny of the spatial memory capability in rats. Study 1 aimed at evaluating the developmental trajectory of spatial memory by using the object-place recognition task. Study 2 was designed to further understand how early infant rats use spatial information to drive spatial behavior. Study 3 was designed to unveil the effect of early spatial experiences in the adult spatial memory capability. Results of Study 1 showed that spatial memory was first expressed during the infantile period as familiarity preference, while the adult-like expression of the memory (i.e., novelty preference) was exhibited during the adolescence period without further changes. The object-place recognition task was unable to validly assess spatial memory capabilities in early infant pups. By using a simple spatial habituation task, study 2 showed that rats were able to form persistent spatial representations already during early infancy. Lastly, the results of Study 3 showed that rats subjected to spatial experiences during infancy had an enhanced spatial memory performance in adulthood. This enhanced effect was experience-specific, context dependent, restricted to the infantile period, and it required the occurrence of sleep after the infantile experiences. Furthermore, infantile spatial experiences increased neuronal activity in the prelimbic (PL) region of the medial prefrontal cortex, a key area involved in the processing of schema information. Three major conclusions can be drawn from the present thesis. First, rats are able to form spatial representations and use it to drive behavior from early infancy. Second, the expression of spatial memory is dynamic and change across ontogeny and third, the adult spatial capability is built upon early spatial experiences.

Synopsis

1 Introduction

1.1 Spatial memory in adulthood

Remember important locations, such as where to find shelter and food, is among the most fundamental of our cognitive capacities. Over a century of behavioral and brain research on spatial navigation in humans and animals has greatly increased the understanding of this cognitive capacity in adulthood. At the brain level, it has been well established that spatial representations are supported by the hippocampus and related limbic areas (O'keefe et al., 1998; Maguire et al., 1999; Moser et al., 2015). At the behavioral level, a great number of tasks have been developed to uncover the relevant spatial features that govern the capacity to navigate through space (Paul et al., 2009; Vorhees and Williams, 2014).

1.1.1 Allocentric and egocentric spatial representations

From a cognitive point of view, there are at least two distinct types of navigation; egocentric and allocentric. Egocentric navigation is characterized by the ability to navigate using proximal cues related to the self like turns (e.g., left-right) or signposts. The allocentric navigation employs elements in the environment regardless of the own position. It is the ability to recall and recognize landmarks (e.g., distal cues) to finally be able to create mental maps of the surrounding environment (Burguess et al., 2002; 2006). Allocentric spatial representations are strongly supported by the hippocampus while egocentric representations are not (Morris et al., 1982; Eichenbaum et al., 1990; Packard and McGaugh, 1996). This was nicely observed in an experiment using the Morris water maze task in rats. In the study, rats were trained to navigate in a pool to find a platform that allowed them to escape from the water. Rats that relied on distal cues (i.e., allocentric navigation) could not find the platform after receiving a lesion in the hippocampus, while rats that relied on proximal cues were not affected (Morris et al., 1982; Eichenbaum et al., 1990). Similar results were found by using other spatial tasks such as the spatial recognition tasks and the T-maze (Barker and Warburton, 2011; Pckard and McGaugh, 1996).

1.1.2 Spatial memory as an episodic memory component

The representation of the space is a fundamental pillar of episodic memory. Episodic memory is the memory for personally experienced events, it contains information about what happened, where and when (Tulving, 1993; Eichenbaum and Cohen, 2001). Although it was first thought of as a type of memory proper to humans, later studies revealed that this memory is also present in non-human animals and it depends on the intact hippocampal function (Clayton and Dickinson, 1998; DeVito and Eichenbaum, 2010; Allen and Fortin, 2013). In the rat model, the item ("what"), the spatial ("where") and the temporal ("when") component of the episodic-like memory can be individually evaluated by using variations of the object recognition paradigm (Eacott and Norman, 2004; Kart-Teke et al., 2005; Dere et al., 2006). Object recognition tasks exploit the natural tendency of adult rodents to explore novelty (Berlyne, 1950; Dix and Aggleton, 1999). As a great advantage, recognition tasks prescind from elements associated with other memory systems such as reward training or exposition to appetitive/aversive stimulus like a pool full of water or electric shocks.

The object-place recognition (OPR) task has been used to study the spatial component of the episodic-like memory (Inostroza et al., 2013). It consists of an encoding phase during which the animal can explore two objects in an arena. After a retention interval, the animal return to the same arena where one of the objects has been displaced to a new location. Spatial memory is inferred from the increased exploration toward the object in the novel location (Ennaceur and Meliani, 1992). It has been shown that the expression of the immediate-early gene c-fos (used as a marker for neuronal activity) in the dorsal hippocampus of the rat increases and positively correlates with the OPR memory performance (Mendez et al., 2015). Moreover, it has been shown that the OPR memory requires an intact hippocampus and post-encoding sleep for its consolidation (Barker and Warburton, 2011; Inostroza et al., 2013; Sawangjit et al., 2018). Other variations of the object recognition task that integrate contextual or temporal information also rely on the intact hippocampus, while the item related information does not (Langston and Wood, 2010; Barker and Warburton, 2011; 2020; but see Sawangjit et al., 2018).

1.1.3 The hippocampal role in episodic memory consolidation and spatial representations

The memory consolidation process explains how memories are transferred from short-term to long-term storage. This process occurs at a synaptic level within the first minutes to hours after learning, and at a system level which takes much longer and involves the reorganization of the brain circuits that support memory (Dudai, 2004). There are several memory systems in the brain that can be distinguished according to the different kinds of information they process and its associated brain structures (White and McDonals, 2002; Squire, 2004; Dudai 2004; 2012). The hippocampal system supports the consolidation of declarative memories (Eichenbaum, 2000). Declarative memories can be separated into two types; semantic (memories for general knowledge or schemas) and the already mentioned episodic memory (Eichenbaum and Cohen, 2001).

The standard theory of memory system consolidation states that episodic memories first depend on the hippocampal function but with time, these memories are transformed into a schema-like representation that prescinds from contextual details. The schema or gist representation is transferred to neocortical structures such as the medial prefrontal cortex (mPFC) which is thought to work as long-term storage (Winocur and Moscovitch, 2011; Sekeres et al., 2018). The memory transformation process depends on repeated explicit retrievals of the experience (e.g., being exposed to several recall phases or to similar experiences) or implicit retrievals (e.g., memory replay during sleep) from which the schema derivates (Born and Wilhelm, 2012; Dudai et al., 2015). Ample evidence has shown that post-encoding sleep does not simply strengthen representations but qualitatively transform memories in a way that enhances specific gist aspects (Diekelman and Born, 2010; Inostroza and Born, 2013; Klinzing et al., 2019).

The hippocampus and the neocortex interact to serve the system consolidation via multiple pathways that support the bidirectional exchange of information. The prelimbic (PL) region of the mPFC, hippocampal areas (e.g., CA1, CA3 and DG), perirhinal cortex (PRC), the lateral entorhinal cortex (LEC), and the reunions nucleus of the thalamus (RE) form part of those pathways (Eichenbaum, 2017). Several lines of evidence indicate that the hippocampus and the medial prefrontal cortex have a complementary role in the processing of episodic information. The hippocampus is crucial for organizing memories within a spatial and temporal context, while the mPFC plays a fundamental role in the retrieval of context-appropriate memories (Schieller et al., 2015; Ekstrom and Ranganath, 2017; Eichenbaum, 2017).

But how the hippocampus is able to organize the memories within a spatial context? More than 50 years of research have supported the view that the hippocampus and neighboring areas hold the brain representation of space via various spatially-modulated cells. O'keefe and Nadel (1978) showed cells in the hippocampus that fired preferentially when the animals are in a specific spatial location, the place cells. Thereafter, Moser and colleagues discovered cells in the entorhinal cortex that fire in a continually repeating hexagonal grid of fields across the surface of an animal's environment, the grid cells (Hafting et al., 2005). In addition to the place and grid cells, many other cell types have been discovered, such as head-direction and border cells, that form part of the neural system supporting spatial representations in the adult brain (Grieves and Jeffery, 2016; Moser et al., 2017).

In conclusion, nowadays it is quite understood which are the spatial features that the animal use to drive spatial navigation, how the spatial representations are transformed to serve episodic memories, and what are the brain mechanisms supporting these processes. However, all this knowledge has been acquired based on adult cognition. Less is known about how the spatial cognitive capacity change across ontogeny. In the following sections, relevant findings in the field of spatial memory development in humans and rodents will be presented, preceded by a brief description of the similarities between human and rat developmental milestones.

1.2 Comparison between rats and human development

Although the developmental time scale of the human differs from the rat, they share behavioral and neurodevelopmental milestones that allow comparing maturational stages (Semple et al., 2013; Sengupta, 2013). In the rat, the brain growth spurt peaks at post-natal day (PD) 8 and continues until the second post-natal week, corresponding to the early infancy of the human (from birth to 2-3 years) (Watson et al., 2006). By the end of the third post-natal week and the beginning of the fourth, there is a peak in synaptic density, myelination rate, and several changes in neurotransmitters and neuroreceptors, that in the human occurs by the end of infancy period (2-3 years). Around PD25-35 there is a peak in gray matter and cortical thickness that can be observed in the early childhood of the human (4-11 years), accompanied by an increase in sociability and the development of the inhibitory control (Tsujimoto, 2008). Between PD35 and PD48 the rat's

brain exhibits a reduction in synaptic density and the adolescence-type behaviors (e.g., high-risk behaviors) emerge together with the sexual maturity. In the human, this period corresponds to 12-18 years old. By ~PD84 rats already exhibit adult-like characteristics in terms of brain size and behavior similar to ~20 years in humans (Semple et al., 2013). Based on above and according to the developmental trajectory of the hippocampus and its function in memory processes (see below), the different developmental stages of the rat will be referred to as; early infancy (PD15-PD16), infancy (PD17-PD24), early-childhood (PD25-PD31), peri-adolescence (PD31-PD37), adolescence (PD38 and PD48) and young adulthood (~PD84).

1.3 Spatial memory in early life

1.3.1 The emergence of allocentric and egocentric spatial representations

Behaviorally, newborns have a rudimentary capacity to keep track and compensate for their own movements in space from very early in life, which improves dramatically with the development of locomotion (Loewen et al., 2005; Loureco and Frick, 2014). Different forms of spatial navigation are already present in the rat pup about PD13-15, which is temporally close to the eyes opening. Locomotion and explorative behavior increase progressively through PD21, age when pups do not rely on the mother for survival, also known as the weaning day (Tan et al., 2017). The capacity to navigate the environment based on spatial representations change across development (Lourenco and Frick, 2013). Studies in rodents and humans have suggested that egocentric based navigation emerge earlier than allocentric navigation (Rudy et al, 1987; Brown et al., 2000; Akers et al., 2007; Ribordy et al., 2013; Ribordy Lambert et al., 2015).

In humans, the ability to navigate using allocentric information is present by 3 years of life which continues to develop in an experience-dependent manner (Ribordy et al., 2013; Fernandez-Baizan et al., 2019; Newcombe, 2019). Similarly, the capability to form allocentric spatial representations in rodents emerges around PD20-21 when the spatially-modulated cells become functional and continue developing until reaching the adult-like function in the adolescent period (Tan et al., 2017). The spatially-modulated cells mature along different temporal trajectories. Head-direction cells emerge first around PD12, even before the eyes opening (Langston et al., 2010; Tan et al., 2015). Although the internal dynamics of the cell firing are adult-like at PD12 (Basset et al., 2018),

the stability and reliability of the directional signal of the head-direction cells improve dramatically after the eyes are open, reaching the adult level during early infancy (Tan et al., 2015). Place cells emerge later around PD15 with a spatial accuracy restricted to the borders, which is extended to the entire environment after weaning (Muessig et al., 2015). Place cells continue developing until reaching the adult-like spatial precision during adolescence (Scott et al., 2011). Grid cells emerge last around the age of weaning and rapidly reach the adult level (Wills et al., 2010). Importantly, place and grid cells are thought to hold the allocentric spatial representations in the adult brain (Jin et al., 2020), and coincidentally are the last ones to reach the adult-like functioning in the rat's ontogeny at around PD38. Thus, the delayed adult-like appearance of the place and grid cells have served as a possible explanation for the delayed emergence of allocentric representations (Tan et al., 2017).

1.3.2 The infantile episodic memory

It is well established in adults that the hippocampus plays a role in recognition memory when such memory has a spatial or a temporal component (Barker & Warburton, 2011; Mendez et al., 2015). Whereas the item component, usually evaluated with the novel-object recognition task, is processed mainly by the perirhinal cortex (Barker and Warburton, 2011). In the rat's ontogeny, the item and place components of the episodic-like memory emerge at different time points. The ability to encode the item information emerges first around PD17 (Jablonski et al., 2013; Westbrook et al., 2014), followed by the ability to associate an item identity with the context where it was presented (Ramsaran et al., 2016). The place component emerges later around PD21 when rats can recognize whether an object has been displaced or not (Westbrook et al., 2014). At PD30, rats can associate an item's identity with its location by remembering precisely which object has been in which location (Ainge and Langston, 2012). Importantly, the capacity to consolidate spatial and contextual information emerges much later than the capacity to merely encode it (Carman et al., 2001; Akers et al., 2012; Westbrook, et al 2014; Ramsaran et al., 2016). It is thought that the distinct trajectory of the capacity to process item and place information underlies the extended maturation of different hippocampal circuits (Lavenex et al., 2007; Donato et al., 2017). The complete maturation of the hippocampus and its circuits has been associated with the emergence of episodic memory which occurs after the infantile period (Newcombe et al., 2007; Lavenex and Banta Lavenex, 2013; Gomez and Edgin, 2015).

The shared impression of being unable to recall episodic memories from early life has intrigued psychologists for decades (Freud, 1953; Bauer, 2006; 2007). The concepts of infantile and childhood amnesia respectively denote the observation that experiences from the first 3 years of life are largely forgotten, and memories from the years 3–8 are sparse (Howe, 2019). Theories from psychology associated these phenomena with the development of complex cognitive processes such as the self-concept, language, and the theory of mind (Nelson and Fivush, 2004). However, recent studies revealed that the apparent inability to form long-term memories based on early life events does also occur in rodents (Akers et al., 2012; Josselyn and Frankland, 2012; Alberini and Travaglia, 2017). Impressively, these studies in rodents have shown that episodic memories from early life are actually not totally forgotten, as they can be recovered with the presence of a reminder or by reactivating the hippocampal engrams involved in its encoding during infancy (Travaglia et al., 2016; Guskjolen et al., 2016; 2018). Thus, instead of being forgotten, infantile episodic memories are store as a latent memory trace until circumstances are suitable for its manifestation (Alberini and Travaglia, 2017).

1.3.3 The development of the hippocampus and the effect of early life experiences

The inability to recollect episodic memories from early life stands in stark contrast with the influence that these events have in adulthood. It has been shown in human and animal studies that early experiences have a tremendous impact on brain development and adult behavior (Tang, 2001; Hensch, 2005; Hofer et al., 2006; Lupien et al., 2009; Zeanah et al., 2009; Perry and Sullivan, 2014). There are specific time windows during early-life when the brain's plasticity is maximal and if sensory experience is abnormal or absent, it can impair sensory representations or be nearly impossible to learn certain skills later in life (Cisnero-Franco et al., 2020). Those developmental time windows are known as critical periods.

During infancy, the hippocampus undergoes a developmental critical period similar to those implicated in the development of vision, language learning, and familial imprinting (Lorenz, 1935; Wiesel and Hubel, 1963; Kuhl, 2004; Alberini and Travaglia, 2017). Hippocampal circuits mature late compared with other cortical areas, with distinct regions maturing at different rates (Lavenex et al., 2007; Donato et al., 2017; Gomez and Edgin, 2016). The infantile hippocampus is characterized by distinct conditions of synaptic plasticity, increased neurogenesis and experience-dependent functional maturation (Mullally and Maguire, 2014; Travaglia et al., 2016; Alberini and

Travaglia, 2017; Bessieres et al., 2020). These distinct conditions are thought to underlie the paradoxical observation that early experiences that seem to be forgotten have long lasting influences on adult behavior (Mullally and Maguire, 2014; Travaglia et al., 2016; Alberini and Travaglia, 2017; Bessieres et al., 2020).

1.4 Research questions

Over a century of behavioral and brain research on spatial navigation in humans and animals has greatly increased the understanding of this cognitive capacity. Summarizing what was mentioned above, there are at least two types of navigations, egocentric (based on proximal cues) and allocentric (based on distal cues). Spatial memory, specially allocentric representations are considered a fundamental component of episodic memory. In rats, the spatial component of the episodic-like memory can be evaluated by using the OPR task, which has been shown to be hippocampal-dependent. The hippocampal role in the system consolidation of episodic memories (i.e., long-term storage) is to organize the episodic information within a spatial and temporal context. All this knowledge about spatial cognition has derived from studies conducted in adults. Much less is known about the spatial capability across ontogeny, specifically from infancy to adulthood.

Developmental studies have shown that the spatial capability change across ontogeny. The accepted view is that an immature form of spatial cognition is present during early infancy and progressively evolve until reaching an adult-like functioning. The evaluation of the spatial component of the episodic-like memory across ontogeny is scarce. The studies done using the object-place recognition task were restricted to early developmental stages (e.g., infancy or early-childhood) (Westbrook et al., 2014; Travaglia et al., 2018) or they used short retention intervals (Krüger et al., 2012). Therefore, how do rats form a persistent one-trial spatial memory across ontogeny and when do they exhibit an adult-like performance remain elusive. On the other hand, the inability of infantile rats to form long-term spatial and episodic memories has been attributed to the distinct condition of the hippocampus during infancy. Despite the evidence showing that the hippocampus undergoes a developmental critical period during infancy, no studies have been conducted to unveil the impact that infantile hippocampal-dependent spatial experiences exert on the adult spatial memory capability.

In light of the protracted development of the hippocampus and the hippocampal functions, the present thesis aims to study the ontogeny of the spatial memory capability across ontogeny, from infancy to adulthood, and the impact that infantile spatial experiences exert on adult behavior.

2 Summary study 1

Study 1 was designed to elucidate the ontogeny of the spatial memory capability in rats by using the object-place recognition (OPR) task at different post-natal ages, i.e., early infancy (PD15), infancy (PD18), early-childhood (PD25), peri-adolescence (PD31), adolescence (PD38 and P48), and young adulthood (PD84). The encoding phase of the OPR task comprised 5-min during which rats could explore two objects. After a retention interval of 3 hours, rats were returned to the arena for a 5-min recall test. This time, one of the objects was placed in a novel location. 3 h of retention interval were used to evaluate a persistent spatial memory. Memory was quantified through an object-location discrimination index. A positive discrimination index denotes an exploration preference for novelty whereas a negative discrimination index denotes a preference for familiarity. Other behavioral measurements were used as complementary data, e.g., time spent in different arena zones, distance travelled, object latency, and total object exploration.

Results showed that rats at PD18 and PD25 exhibited a significant preference for the object at the familiar location. The PD31 group showed a null preference while the remaining groups (PD38, PD48 and PD84) showed a significant preference for the object at the novel location (Fig. 2, appended paper 1). Because young rats, particularly in the early infancy age (PD15), did not show full-blown object exploration (Fig. 3A, appended paper 1), we investigated whether a discrimination index based on the time spent in the zone of the arena containing either the familiar or novel object-location might be more sensitive to reflect object-location memory than the actual object exploration. This analysis was restricted to the younger groups PD15, PD18, PD25 and PD31. Rats at PD15 showed no preference for either zone. Consistently with the familiarity preference observed in the exploration towards the objects, PD18 and PD25 groups spent more time in the zone corresponding to the familiar object-location. Interestingly, the PD31 group also exhibited a preference for the zone that contained the familiar object-location (Fig. 4, appended paper 1). Further analyses were performed to examine whether pups at PD15 displaced any form

of memory-based navigation in the arena (Fig. 5, appended paper 1). Results showed no sign of memory-based navigation.

In conclusion, the data of Study 1 indicate that rats from infancy (PD18) and onwards are capable of forming a persistent spatial memory. Interestingly, this memory was initially expressed as preferential exploration for the object at the familiar location. This expression gradually changed in the course of development to a null preference in PD31 and later to a novel location preference that started at PD38 and continued until young adulthood. We did not find any behavioral sign of spatial memory capability before PD18 when using the OPR task. Since PD15 rats did not explore the objects, remains unclear whether early infant rats are unable to navigate based on persistent spatial memory or simply the OPR task is not suitable to test this capability in early infant rat pups.

3 Summary study 2

In study 1 it was shown that rats did not explore the objects long enough to test spatial memory with the OPR task before PD18. This makes such object-based tasks unreliable for testing spatial capabilities before this age. Study 2 was designed to evaluate whether early infant pups can use spatial memory to navigate by using a simple spatial habituation task.

Habituation is a basic form of learning which describes the progressive decrease of the behavior due to the repeated exposure to a stimulus (Domjan, 2002). The spatial habituation task relies on the pup's capability to navigate the environment through locomotion. The task was conducted on PD16 and consisted of two habituation sessions in which pups were placed in an arena with the same spatial configuration each time. After ~140 minutes, rats were returned to the arena but with different spatial conditions depending on the group. The No-change group was returned to exactly the same spatial environment (i.e., with the same proximal and distal cues), while a Prox-Dist group was returned to a different spatial environment (i.e., with different proximal and distal cues configurations). Two further groups were used to disentangle the contribution of proximal and distal cues in the memory-based navigation (Fig. 1A, appended paper 2).

The results showed that the locomotor activity (i.e., distance travelled in the arena) significantly decrease from the first to the second habituation session, meaning that all rats were able to

habituate to the spatial environment equally. At the test trial, No-change group showed a further decrease in locomotion, while Prox-Dist group increased the locomotion. The group that returned to an environment that only differed in the proximal cues (i.e., Prox group) also showed an increase in locomotion compared to the group that returned to the no-changed environment. On the other hand, the group that was returned to an environment with novel distal cues (i.e., Dist group) behaved similarly to the group that returned to the no-changed environment (Fig. 1C-D, appended paper 2).

In conclusion, Study 2 showed that early infant rats are indeed capable of forming a persistent spatial memory that mainly relied on proximal cues to drive spatial navigation.

4 Summary study 3

Study 3 was designed to investigate how early spatial experiences during the hippocampal critical period (i.e., infancy) affect the adult spatial capability. This study explored whether the effect of infantile spatial experiences was context specific, restricted to a particular developmental period, and sleep dependent. Additionally, the brain areas relevant for the consolidation of episodic and spatial memories were evaluated by measuring the c-fos activity in the adult brain.

To induce spatial experiences during infancy, the rats of the group Spatial-experience were exposed to the OPR task for 4 days during infancy (at PD18, PD20, PD22 and PD24). A control group of rats was exposed to a non-spatial (hippocampal-independent) task during infancy by only changing the kind of one of the two objects instead of changing its spatial configuration. An additional No-experience group did not undergo any manipulation during infancy. To evaluate the adult capability to form spatial representations, all groups were tested in the OPR task at ~PD84. 3 hours delay between encoding and retrieval was used to assess a persistent spatial memory capability. The object-location discrimination index was used to quantify spatial memory. 90 min after the adult OPR retrieval test the rat's brains were removed and processed for c-fos quantification and analysis.

Results showed that, in the adult OPR retrieval test, only the Spatial-experience group showed an enhanced spatial memory that was significantly better than the two control groups (i.e., Non-spatial

experience and No-experience groups) (Fig. 1B, appended paper 3). This enhanced performance was consistent across the 5-min of the adult retrieval test (Fig. S2, appended paper 3), which contrasted with the transitory memory expression of the No-experience group that was restricted to the 2nd and 3rd minute of the retrieval test. The Non-spatial experience group showed no memory. At the brain level, the Spatial-experience group showed increased c-fos activity in the PL region of the mPFC and a decreased activity in posterior areas such as the parietal and perirhinal cortex, and the reunions nucleus of the thalamus. No differences were found in hippocampal areas (Fig. 2B, appended paper 3).

We further asked whether the enhanced spatial memory capability observed in the Spatial-experience group resulted from the episodic representation of the discrete spatial events that persisted into adulthood. To answer this question, we tested a Long-term OPR group that underwent a single OPR encoding phase at PD24 and the retrieval test in adulthood. Results showed that at this remote retrieval test rats performed at chance level and significantly different from the Spatial-experience group during the first minute of the test phase. Curiously, analysis of the entire 5 minutes retrieval test revealed that starting from 2nd minute, rats displayed a significant preference for the object in the familiar location (Fig 1C and Fig. S3, appended paper 3).

Next, we hypothesized that the beneficial effect might have been driven by a schema-like representation constructed upon the early spatial experiences. Because a schema-like representation is in theory context independent, we performed a group of rats that followed the same infantile conditions as the Spatial-experience group but in the adulthood, the OPR test was performed in a completely different context. The results showed that this context-change group was incapable of forming a stable spatial representation. The context-change group's performance was significantly worse than the Spatial-experience group which did not undergo contextual change (Fig 1C, appended paper 3).

In light of the hippocampal critical period, we wondered whether infancy was a period of special sensitivity to spatial experiences. To answer this question, the effect of early spatial experiences was tested at two further ages i.e., Early-childhood (PD25 to PD31) and Adolescence (PD48 to PD54) followed by the same OPR test in adulthood. No differences were found between the Early-childhood and Adolescence group in the adult OPR test. Actually, their performance was

indistinguishable from the No-experience control group, showing a transitory memory expression at the 3rd minute of the retrieval test. Furthermore, their performance was significantly worse than the Spatial-experience group which was subjected to spatial experiences during the infantile period (Fig. 4A, appended paper 3).

Finally, we asked whether the beneficial effect of early spatial experiences depends on the occurrence of sleep after the infantile experiences. To answer this question, a group of rats was exposed to spatial experiences during infancy followed by 90 minutes of sleep deprivation. As for the other groups, the sleep-deprivation group was tested on adulthood in the OPR task. Sleep deprived rats did not profit from the infantile spatial experiences. Additionally, we evaluated the c-fos activity after the adult retrieval test. The results revealed a decreased activity in the PL region compared with the Spatial-experience group, while hippocampal activity remained unchanged (Fig. 4B, appended paper 3).

In conclusion, this series of experiments revealed that the exposure to discrete changes in spatial configurations during infancy strongly affects the adult capability to form a persistent spatial memory, while a single spatial episode itself is not retrievable. This effect is context-dependent, restricted to the infantile period and also depended on the occurrence of sleep after the infantile spatial experience. Furthermore, results from the c-fos activity suggest that the effects of infantile spatial experiences might reside in the PL rather than in hippocampal areas.

5 Discussion

5.1 Changes in memory expression across ontogeny

In study 1 we evaluated the developmental trajectory of spatial memory capabilities by using the object-place recognition (OPR) task at different post-natal days. Results showed that PD18 was the earliest age when pups showed a memory driven spatial navigation which was expressed as a preferential exploration towards the object in the familiar location. Also, we found that the expression of the spatial memory progressively changed until it reaches the regular novelty preference at PD38 without further changes in development. Interestingly, the age of PD38 coincides with the time when both, the hippocampus and the brain machinery involved in the

formation of allocentric spatial representations such as place and grid cells are adult-like (Ainge and Langston, 2012; Wills et al., 2013; Tan et al., 2017).

Few studies have also investigated when the OPR memory emerges in rodents. The earliest memory expression found using a comparable retention interval was at PD24 in rats (Westbrook et al., 2014; Travaglia et al., 2018), 6 days later than the age found in study 1. When using shorter retention intervals (1 to 10 min), the emergence of spatial recognition memory in rats also variates between studies, the earliest being found at PD16 (Krüger et al., 2012; Jablonski 2013; Westbrook et al 2014). The high variability in terms of experimental design, rodent species, strain, arena/object types, behavioral analyses, habituation sessions and retention intervals, could explain the differences in the post-natal day when this capability first emerge (Cruz-Sanchez et al 2020). Although the present study and studies from other labs share some procedural differences, none of the previous studies have found familiarity preference as it was found here on PD18 and PD25.

What could drive a familiarity preference? An intuitive answer would be that our younger rats had greater levels of anxiety, as some studies showed that younger rats require longer habituation sessions than adult rats (Feigley et al 1972; File, 1978). This hypothesis was ruled out by analyzing behavioral indicators of anxiety (i.e., the proportion of time the animals spent in the center of the arena vs near to the border during the retrieval phase). Results revealed comparable values for the younger (PD18 and PD25) and the oldest groups (PD84 which expressed novelty preference). Moreover, study 1 stands out for the increased handling and habituation sessions (known to reduce stress) compared to other studies that have found novelty preference in the OPR task at similar ages (Cruz-Sanchez et al., 2020). That is why it seems rather improbable that the familiarity preference observed in study 1 was driven by anxiety.

Unlike rodent studies, the developmental shift in the exploratory preference from familiarity to novelty is a well-known phenomenon in human literature (Rose et al., 1982; Houston-Price and Nakai, 2004; Perone and Spence, 2013). Human infants are more likely to prefer a familiar stimulus earlier in life when they have had insufficient familiarization (Richmond et al, 2014), when the stimulus is complex rather than simple (Hunter and Ames, 1988), when the task involves cross-modal transfer of information (Wagner and Sakovits, 1986) or when the stimulus belongs to a conceptually relevant category (Border et al., 2002). Based on that, it has been proposed that

familiarity preference in early life is due to a weaker or incomplete representation of the familiar stimulus that promotes further exploration until it is sufficiently strong to move on to the novel stimulus (Shinskey and Munakata 2005, 2010). It is tempting to speculate that similar phenomena might have driven the familiarity preference observed in the PD18 and PD25 groups. If that is the case, then why PD18 and PD25 rat pups formed an incomplete spatial representation? A possible answer for this question might reside in the slower development of the allocentric system in the rat.

It has been proposed that spatial navigation based on egocentric representations emerges earlier than allocentric (Siegel and White, 1975). Studies in the Morris water maze have helped to further understand the determinants of egocentric and allocentric navigation in infant rats. It was shown that infant rats were first able to find the platform using proximal cues, but only a couple of days later, pups were able to find the platform based on distal cues (Akers et al., 2007; Rudy et al, 1987; Brown et al., 2000). However, Carman and Mactutus (2001) found that allocentric spatial navigation can also be found earlier (at PD17) when the size of the pool was adjusted to the pup's size (Carman and Mactutus, 2001). This evidence tells us that rat pups can navigate based on allocentric information as young as they do using egocentric information and that the capacity to express early allocentric learning is sensitive to the test conditions such as the size of the pool.

On the other hand, it has been shown that the full capacity to navigate using allocentric reference continues developing after its emergence. This was demonstrated in rats by using two variants of the allocentric version of the Morris water maze. Rats pups were trained to find the hidden platform in a precise location in the pool (regardless of the room position) or in a precise location in the room (regardless of the pool position). The last variant supposes an extra allocentric difficulty since rats cannot rely on the spatial relationship between the platform and the inner-pool distances to drive navigation. Results showed that pups at PD20 were able to find the platform in the precise pool location, but do not in the room location, which only emerged later at PD27 (Akers et al., 2009). In agreement with the progressive development of the allocentric spatial representation observed in rats, studies in human infants have shown that a rudimentary form of allocentric representation emerges within the first year of life although it is not as strong as it becomes later (Newcombe 2019).

The above paragraphs tell us that both representational systems, egocentric and allocentric, might develop in parallel (Burges, 2006), and that the allocentric system continues developing presumably until PD38 (Ainge and Langston, 2012; Wills et al., 2013; Tan et al., 2017). Unlike previous developmental studies in the OPR task, study 1 thought to promote allocentric navigation by introducing the rat to the arena from different places and by prescinding from proximal cues inside the arena (Whishaw et al., 1986; Langston and Wood, 2010). Thus, it is reasonable to think that rats pups at PD18 or PD25 formed an incomplete allocentric spatial representation because the allocentric system on which pups relied to perform the OPR task was still under maturation (Shinskey and Munakata 2005, 2010; Ainge and Langstone, 2010; Wills et al., 2013; Tang et al., 2017). It would be interesting to test whether by allowing longer periods of encoding or by providing proximal cues inside the arena, pups would be able to form a complete spatial representation and therefore show novelty preference.

Another plausible explanation for the familiarity preference might be related to the observation that infant rats cannot form long-term memories based on a single trial (Akers et al., 2014; Guskjolen et al., 2017; Alberini and Travaglia 2018; Bessieres et al., 2020). In this context, rather than an incomplete representation, rats could have formed a full allocentric representation, but after 3 hours, some information was lost encouraging the animals to further explore the familiar object-location. We ruled out this hypothesis by performing an extra group of rats at PD18 which was subjected to exactly the same OPR task but with a shorter retention interval of only 5 minutes. The data confirmed previous results. Rats showed preferential exploration toward the object in the familiar location. This evidence discarded post-encoding forgetting as an explanation of the familiarity preference.

An intriguing finding is the null preference expressed by the group tested on PD31. Interleaving periods of memory expression have also been found in human developmental studies (Koski et al., 2004; Richmond and Power, 2014;). In the case of study 1, rather than a random preference, the PD31 group stands out by an increased variability, including animals with a high preference for novelty while others for familiarity. Supporting this idea, further analysis of the time spent in different zones of the arena revealed that that rats at PD31 preferred to stay longer periods in the zone containing the object in the familiar location. Thus, the high variability observed in the PD31

group's performance could speak for conflicting preferences among the group where the null preference reflects an artifact of grouping data rather than a lack of memory (Roder et al, 2000).

Another important finding was that early infant pups did not fulfill the minimum object exploration required to evaluate memory with the OPR task. As an alternative, we explored the time the rats spend in different zones of the arena instead of just looking at the object exploration. These analyses did not evidence any sign of memory-based spatial navigation in early infant pups. However, being unable to find a behavioral expression of spatial memory in PD15 rats does not exclude the possibility that these younger rats are indeed capable of forming spatial representations.

5.2 A behavioral test for a memory-based spatial navigation in early infant rats.

By using a simple spatial habituation task, study 2 revealed that early infant rats are indeed capable of forming a spatial representation that lasted for at least ~140 min. This conclusion is inferred from the fact that rats exposed to the same spatial environment showed a decrease in locomotion (i.e., decrease in the distance travelled in the arena), while rats that returned to a novel environment showed an increase in locomotion. In combination, these results indicate that early infant rats formed a spatial representation that was used to differentially regulate exploratory behavior in a familiar versus a novel spatial environment.

To my knowledge, this is the first experiment showing that rats at PD16 are able to navigate based on a persistent (~140 min) spatial memory, contradicting previous findings claiming that young rats are able to encode, but unable to retain for a long time the contextual or spatial information (Akers et al., 2012; Westbrook et al 2014; Travaglia et al 2018; Sanders et al., 2020). This speaks in favor of the idea that being able to observe and evaluate spatial capabilities across development critically depends on the task conditions and the pup's behavioral repertoire (Carman et al., 2001; 2003). This idea is also extended to developmental studies in humans (Jones et al., 2011; Edgin et al., 2019).

Furthermore, study 2 shows that the increased locomotion in a novel spatial configuration relies mainly on proximal and does not necessarily on distal cues. These results suggest that, when

available, proximal cues will primarily drive spatial navigation during early infancy (Siegel and White, 1975; Rudy et al, 1987; Brown et al., 2000; Akers et al., 2007) which agrees with the classic idea that egocentric navigation emerges earlier than the allocentric (Piaget and Inhelder, 1956). However, our results do not exclude the possibility that infant pups are able to process distal information. Actually, work from Ramsaran and colleagues (2016) showed that early infant pups were able to encode objects-context association including information about distal cues (Ramsaran et al., 2016) supporting the idea that pups are able to process allocentric spatial information from early on.

5.3 The effect of early spatial experiences on adulthood.

About 70 years ago Piaget postulated that the representation of space, as many other cognitive processes, is build up upon the active interaction between the subject and its environment (Piaget and Inhelder, 1956). Results of study 3 support Piaget's premise by showing that discrete spatial experiences (such as being exposed to an object that was changed from its location) during infancy builds the adult spatial capability.

Precisely, we showed that being exposed to spatial experiences during infancy, which is known to be hippocampal dependent, strongly enhanced the adult spatial capability while infantile non-spatial experiences (or non-hippocampal dependent) did not produce such enhancement. In agreement, recent findings have shown that hippocampal dependent learning experiences during infancy biologically mature the hippocampus by producing unique molecular, cellular, and synaptic maturational changes, while non-hippocampal dependent learning did not produce such biological changes (Bessieres et al., 2020). Results of study 1 suggest that the beneficial effect of infantile hippocampal-dependent experiences does also persist until adulthood.

It has been shown that rats cannot form long-term memory based on discrete episodes when encoded during infancy (Travaglia et al 2018; Guskjolen et al 2018). Accordingly, the Long-term OPR control group of study 3 that underwent a single spatial encoding phase during infancy and was tested on adulthood did not express spatial memory during the first minute of the adult retrieval. Impressively, these rats showed a preference for the object in the familiar location from the second minute, suggesting that a rudimentary form of infantile spatial representation was preserved until adulthood. The familiarity preference observed in study 3 nicely match with the

results from study 1 previously discussed. Results of study 1 revealed that rats at PD18 and PD25 expressed object-place recognition memory as familiarity preference. The familiarity preference was interpreted as the effect of an incomplete allocentric spatial representation of the object's location caused by the immature stage of the allocentric system in the rat. The fact that rats of the Long-term OPR group encoded the spatial information at PD24 and expressed familiarity preferences at the retrieval test suggests that the presumable "incomplete spatial representation" was kept until adulthood.

To the question, was the effect of infantile spatial experiences on adulthood based on the memory of discrete episodes? The answer is probably no. Results from the Long-term OPR group showed that the single spatial episode was expressed as familiarity preference, while the group exposed to several spatial experiences during infancy showed an enhanced novelty preference in the adult OPR test. This suggests that being exposed to several spatial experiences upgrades the cognitive capability to form spatial representations rather than forming a memory of the events themselves. Supporting this idea, results of further experiments (Contreras et al., *unpublished data*) revealed that prior spatial experiences in the OPR task can accelerate the adult-like form of memory expression (i.e., novelty preference) at PD24 and PD31 (an age when naïve rats express familiarity or null preference). Importantly, the "acceleration" of memory expression was not achieved when the prior experience in the OPR task lacked its spatial component (i.e., when the objects remained stationary). Thus, in study 3 the infantile spatial experiences might have accelerated and strengthened the adult capability to form spatial representations, which explains the enhanced novelty preference across the 5 minutes of the adult OPR retrieval test exhibited by the spatial experience group.

The maturation of the hippocampus goes along with the development of the capability to form contextually detailed memories (e.g., episodic memories). Thus, in a stage when the hippocampus is still under development memories are more likely to be generalized (Ramsaran et al., 2019). The data from the context-change group revealed that the enhancing effect of the infantile spatial experiences did not generalized to a different context in adulthood. In line with the idea that infantile spatial experiences accelerated the adult form of spatial capability, and also considering that hippocampal-dependent learning during infancy biologically matures the hippocampus (Bessieres et al., 2020), it can be speculated that infantile spatial experiences boosted the

hippocampal-dependent capability to integrate contextual information into the infantile memory. That rats benefited from the infantile experiences on adulthood without being able to form a long-term memory for a single episode suggests that the spatial experiences were transformed into a schema-like memory. The standard system consolidation theory predicts that after a period of time, memories of single episodes are transformed into a schema-like memory that prescinds from contextual details. Results of the context-change group contradict the system consolidation prediction by showing that rats indeed required contextual information to retrieve the (presumable) schema memory built upon infantile experiences.

The mPFC is a brain structure known to be relevant for the ability to access and use schema information (Alonso et al., 2020; Brod et al., 2013). In agreement, the group exposed to infantile spatial experiences expressed an increased c-fos activity in the PL region of the mPFC after the adult OPR test compared to the Non-spatial experiences and No-experience groups. Similar results were found in another study using different immediate-early genes (e.g., Zif268 and Arc) to map brain activity (Tse et al., 2011). In the experiment, adult rats were trained in a place-flavor association task which was previously proved to build a schema memory that facilitates the integration of new task-related information (Tse et al., 2007). Results showed increased levels of Zif268 and Arc in the PL cortex in the rats that were required to integrate new place-flavor associations into the schema. Furthermore, the inhibition of the PL prevented the long-term storage of the schema and the integration of new information into it (Tse et al., 2011). In light of these results, it is tempting to speculate that a similar role might play the increased activity observed in the PL region of the group with infantile spatial experiences, as the PL cortex do also play a role in schema representations formed across ontogeny (Brod et al., 2013). Interestingly, hippocampal c-fos activation turned to be similar among the main groups of study 3, which coincide with the view of the standard system consolidation theory which states that the hippocampus is crucially involved at the beginning of the system consolidation process, but not later (Winocur and Moscovitch, 2011; Sekeres et al., 2018; but see Barry and Maguire, 2020 for a new perspective).

The results of study 3 also showed that the beneficial effect of the early spatial experiences in the adult spatial capability was restricted to the infantile period. The groups that underwent the spatial experiences at later time points, i.e., during early-childhood or during adolescence did not profit from the early spatial experiences as the infantile group. The distinct characteristic of the infantile

hippocampus might account for this effect. At PD17 the hippocampus shows increased levels of synaptic plasticity markers (e.g., immediate early genes, kinases, transcription factors and AMPA receptor subunits) that progressively decay at PD24 reaching levels close to those of adulthood (Travaglia et al., 2016; Tzakis et al., 2020). Concurrently, the hippocampus exhibits an increase in proteins supporting structural and functional maturation, in particular those involved in the growth of dendrites, axons and synapses (Travaglia et al., 2016). The infant hippocampus is also characterized by increased levels of neurogenesis that steadily declines thereafter (Josselyn and Frankland, 2014; Kozareva et al., 2019). All this evidence, together with the fact that most of the adult hippocampal neurons (precisely in the DG) are born during early life (Jabes et al., 2010; Ciric et al, 2019), support the observation of the present study showing that infancy is a period of particular sensitivity to hippocampal-dependent spatial experiences and for taking them to influence adult behavior.

Sleep supports the formation of long-term memories (Diekelman and Born, 2010, Inostroza and Born, 2013; Rasch and Born 2013). The active system consolidation theory claims that during sleep hippocampal representations are reactivated and transferred to cortical areas. In addition, the repeated reactivation of newly encoded events promotes the gradual transformation of memories into persistent schema-like representations (Klinzing et al., 2019). Sleep in human infants has also been found to promote the formation of abstracted memory for objects (Friedrich et al., 2015). Accordingly, results from our sleep-deprivation group showed that sleep after each infantile spatial experience is fundamental for its beneficial effect in adulthood.

Studies in adult brains have shown that the sleep role in memory consolidation relies on a dialogue between the hippocampus and neocortex that occurs during slow-wave sleep through a temporal interaction between neocortical slow oscillations, thalamic spindles and hippocampal ripples (Klinzing et al., 2019; Oyanedel et al., 2020;). In newborn rats, premature communication from the hippocampus to mPFC through oscillatory entrainment has been found already around PD6-PD8 (Haugland et al., 2019) which is converted to bidirectional signaling at PD13-PD15 (Brockman et al., 2011; Kaila, 2011). A hippocampal lesion at birth disrupts this communication, also affecting the performance in the object-place recognition task (Krüger et al., 2012), suggesting an early involvement of the hippocampal-cortical communication in the processing of spatial information. In this context, preventing from sleep during infancy can disrupt the hippocampal-

cortical communication. In support of this idea, it has been shown that sleep plays a crucial role in the early development of the hippocampal system (Del Rio-Bermudez et al., 2020), providing a unique context in which the brain uses an inner mechanism for boosting cortical-hippocampal communication (Del Rio-Bermudez and Blumberg, 2018; Blumberg et al., 2020). Also, there is evidence showing reactivation of spatial information during sleep already during infancy that continues developing until it reaches adult-like patterns by the end of the hippocampal critical period (Farooq and Dragoui, 2019).

All this evidence suggests that the early participation of sleep in the consolidation of hippocampal memories is ruled by similar mechanisms to those found in adulthood (Diekelman and Born, 2010; Huber and Born, 2014; Klinzing et al., 2019). This mechanism might have been critically interrupted in our sleep-deprived pups disrupting the hippocampal-cortical communication which eventually might have led to the schema formation and its transference to cortical long-term storages. c-fos data from this sleep-deprivation group support this view by showing a decrease in the neural activation of the PL cortex compared to the group with undisturbed infantile sleep.

5.4 Conclusions

Three major conclusions can be drawn from the series of experiments conducted during this doctoral thesis: First, rat pups are able to form spatial representations and use it to drive behavior from very early in life. Importantly, the evaluation of the spatial capability during early infancy critically depends on the task demands and the behavioral repertoire of the rats. Second, the expression of the object-place recognition memory changes across development. Memory expression was first observed during infancy as familiarity preference which shifted to the adult-like form of memory expression (i.e., novelty preference) during the adolescence period. This shift in memory expression coincides with the developmental timeline of the brain circuits that support allocentric representations, suggesting that familiarity preference is a rudimentary form of spatial memory that is still under development. Third, the adult capability to form spatial representations is built upon early experiences. Spatial experiences during infancy, and not during later periods, strongly enhance the adult capability to form spatial representations. This effect depends on a series of factors such as contextual similarity and the opportunity of sleep after the infantile experience.

6 References

Aiesel, T. N., & Hubel, D. H. (1963). single-cell responses in striate cortex of kittens deprived of vision in one eye. *J Neurophysiol*, *26*, 1003–1017. https://doi.org/10.1152/jn.1963.26.6.1003

Ainge, J. A., & Langston, R. F. (2012). Ontogeny of neural circuits underlying spatial memory in the rat. *F Front Neural Circuits*, *6*, 8. https://doi.org/10.3389/fncir.2012.00008

Akers, K. G., & Hamilton, D. A. (2007). Comparison of developmental trajectories for place and cued navigation in the Morris water task. *Dev Psychobiol*., *49*(6), 553–564. https://doi.org/10.1002/dev.20227

Akers, K. G., Candelaria-Cook, F. T., Rice, J. P., Johnson, T. E., & Hamilton, D. A. (2009). Delayed development of place navigation compared to directional responding in young rats. *Behav Neurosci*, *123*(2), 267–275. https://doi.org/10.1037/a0014594

Akers, K. G., Martinez-Canabal, A., Restivo, L., Yiu, A. P., De Cristofaro, A., Hsiang, H. L., Wheeler, A. L., Guskjolen, A., Niibori, Y., Shoji, H., Ohira, K., Richards, B. A., Miyakawa, T., Josselyn, S. A., & Frankland, P. W. (2014). Hippocampal neurogenesis regulates forgetting during adulthood and infancy. *Science*, *344*(6184), 598–602. https://doi.org/10.1126/science.1248903

Alberini, C. M., & Travaglia, A. (2017). Infantile Amnesia: A Critical Period of Learning to Learn and Remember. *J Neurosci*, *37*(24), 5783–5795. https://doi.org/10.1523/JNEUROSCI.0324-17.2017

Allen, T. A., & Fortin, N. J. (2013). The evolution of episodic memory. *Proc Natl Acad Sci U S A*, 10379–10386. https://doi.org/10.1073/pnas.1301199110

Alonso, A., van der Meij, J., Tse, D., & Genzel, L. (2020). Naïve to expert: Considering the role of previous knowledge in memory. *Brain Neurosci Adv*, 4, 2398212820948686. https://doi.org/10.1177/2398212820948686

Barker, G. R., & Warburton, E. C. (2011). When is the hippocampus involved in recognition memory?. *J Neurosci*, *31*(29), 10721–10731. https://doi.org/10.1523/JNEUROSCI.6413-10.2011

Barker, G., & Warburton, E. C. (2020). Putting objects in context: A prefrontal-hippocampal-perirhinal cortex network. *Brain Neurosci Adv*, *4*, 2398212820937621. https://doi.org/10.1177/2398212820937621

Barry, D. N., & Maguire, E. A. (2019). Remote Memory and the Hippocampus: A Constructive Critique. *Trends Cogn Sci*, *23*(2), 128–142. https://doi.org/10.1016/j.tics.2018.11.005

Bassett, J. P., Wills, T. J., & Cacucci, F. (2018). Self-Organized Attractor Dynamics in the Developing Head Direction Circuit. *Curr Biol: CB*, *28*(4), 609–615.e3. https://doi.org/10.1016/j.cub.2018.01.010

Bauer ,P. J. (2006). Constructing a past in infancy: a neuro-developmental account. *Trends Cogn Sci, 10*(4), 175–181. https://doi.org/10.1016/j.tics.2006.02.009

Bauer, P. J. (2007). *Remembering the times of our lives: Memory in infancy and beyond*. Lawrence Erlbaum Associates, Mahwah, NJ.

Bessières, B., Travaglia, A., Mowery, T. M., Zhang, X., & Alberini, C. M. (2020). Early life experiences selectively mature learning and memory abilities. *Nat Commun*, *11*(1), 628. https://doi.org/10.1038/s41467-020-14461-3

Blumberg, M. S., Dooley, J. C., & Sokoloff, G. (2020). THE DEVELOPING BRAIN REVEALED DURING SLEEP. *Curr Opin Physiol*, *15*, 14–22. https://doi.org/10.1016/j.cophys.2019.11.002

Born, J., & Wilhelm, I. (2012). System consolidation of memory during sleep. *Psychol Res*, *76*(2), 192–203. https://doi.org/10.1007/s00426-011-0335-6

Brockmann, M. D., Pöschel, B., Cichon, N., & Hanganu-Opatz, I. L. (2011). Coupled oscillations mediate directed interactions between prefrontal cortex and hippocampus of the neonatal rat. *Neuron*, *71*(2), 332–347. https://doi.org/10.1016/j.neuron.2011.05.041

Brod, G., Werkle-Bergner, M., & Shing, Y. L. (2013). The influence of prior knowledge on memory: a developmental cognitive neuroscience perspective. *Front Behav Neurosci*, 7, 139. https://doi.org/10.3389/fnbeh.2013.00139

Brown, R. W., & Kraemer, P. J. (1997). Ontogenetic differences in retention of spatial learning tested with the Morris water maze. *Dev Psychobiol*, *30*(4), 329–341. https://doi.org/10.1002/(sici)1098-2302(199705)30:4<329::aid-dev6>3.0.co;2-q

Brown, R. W., & Whishaw, I. Q. (2000). Similarities in the development of place and cue navigation by rats in a swimming pool. *Dev Psychobiol*, *37*(4), 238–245. https://doi.org/10.1002/1098-2302(2000)37:4<238::aid-dev4>3.0.co;2-j

Burgess N. (2006). Spatial memory: how egocentric and allocentric combine. *Trends Cogn Sci*, *10*(12), 551–557. https://doi.org/10.1016/j.tics.2006.10.005

Burgess, N., Maguire, E. A., & O'Keefe, J. (2002). The human hippocampus and spatial and episodic memory. *Neuron*, *35*(4), 625–641. https://doi.org/10.1016/s0896-6273(02)00830-9

Carman, H. M., & Mactutus, C. F. (2001). Ontogeny of spatial navigation in rats: a role for response requirements?. *Behav Neurosci*, *115*(4), 870–879.

Carman, H. M., Booze, R. M., & Mactutus, C. F. (2002). Long-term retention of spatial navigation by preweanling rats. *Dev Psychobiol*, *40*(1), 68–77. https://doi.org/10.1002/dev.10014

Carman, H. M., Booze, R. M., Snow, D. M., & Mactutus, C. F. (2003). Proximal versus distal cue utilization in preweanling spatial localization: the influence of cue number and location. *Physiol Behav*, *79*(2), 157–165. https://doi.org/10.1016/s0031-9384(03)00089-1

Ciric, T., Cahill, S. P., & Snyder, J. S. (2019). Dentate gyrus neurons that are born at the peak of development, but not before or after, die in adulthood. *Brain Behav*, *9*(10), e01435. https://doi.org/10.1002/brb3.1435

Cisneros-Franco, J. M., Voss, P., Thomas, M. E., & de Villers-Sidani, E. (2020). Critical periods of brain development. *Handb Clin Neurol*, *173*, 75–88. https://doi.org/10.1016/B978-0-444-64150-2.00009-5

Clayton, N. S., Bussey, T. J., & Dickinson, A. (2003). Can animals recall the past and plan for the future?. *Nat Rev Neurosci*, *4*(8), 685–691. https://doi.org/10.1038/nrn1180

Cruz-Sanchez, A., Wilkin, J., & Arruda-Carvalho, M. (2020). Ontogeny of spontaneous recognition memory in rodents. *Neurobiol Learn Mem*, *177*, 107361. Advance online publication. https://doi.org/10.1016/j.nlm.2020.107361

D.E. Berlyne. (1950). Novelty and curiosity as determinants of explorative behavior. *Br. J. Psychol*, 41, 68–80, https://doi.org/10.1111/j.2044-8295.1950.tb00262.x.

Del Rio-Bermudez, C., & Blumberg, M. S. (2018). Active Sleep Promotes Functional Connectivity in Developing Sensorimotor Networks. *Bioessays*, *40*(4), e1700234. https://doi.org/10.1002/bies.201700234

Del Rio-Bermudez, C., Kim, J., Sokoloff, G., & Blumberg, M. S. (2020). Active Sleep Promotes Coherent Oscillatory Activity in the Cortico-Hippocampal System of Infant Rats. *Cereb Cortex*, *30*(4), 2070–2082. https://doi.org/10.1093/cercor/bhz223

Dere, E., Kart-Teke, E., Huston, J. P., & De Souza Silva, M. A. (2006). The case for episodic memory in animals. *Neurosci Biobehav Rev*, *30*(8), 1206–1224. https://doi.org/10.1016/j.neubiorev.2006.09.005

DeVito, L. M., & Eichenbaum, H. (2010). Distinct contributions of the hippocampus and medial prefrontal cortex to the "what-where-when" components of episodic-like memory in mice. *Behav Brain Res*, *215*(2), 318–325. https://doi.org/10.1016/j.bbr.2009.09.014

Diekelmann, S., & Born, J. (2010). The memory function of sleep. *Nat Rev Neurosci*, *11*(2), 114–126. https://doi.org/10.1038/nrn2762

Dix, S. L., & Aggleton, J. P. (1999). Extending the spontaneous preference test of recognition: evidence of object-location and object-context recognition. *Behav Brain Res*, *99*(2), 191–200. https://doi.org/10.1016/s0166-4328(98)00079-5

Domjan, M. (2002). *The principles of learning and behavior*. Stamford: Cenage Learning.

Donato, F., Jacobsen, R. I., Moser, M. B., & Moser, E. I. (2017). Stellate cells drive maturation of the entorhinal-hippocampal circuit. *Science (New York, N.Y.)*, *355*(6330), eaai8178. https://doi.org/10.1126/science.aai8178

Dudai Y. (2004). The neurobiology of consolidations, or, how stable is the engram?. *Annu Rev Psychol*, *55*, 51–86. https://doi.org/10.1146/annurev.psych.55.090902.142050

Dudai, Y., Karni, A., & Born, J. (2015). The Consolidation and Transformation of Memory. *Neuron*, *88*(1), 20–32. https://doi.org/10.1016/j.neuron.2015.09.004

Eacott, M. J., & Norman, G. (2004). Integrated memory for object, place, and context in rats: a possible model of episodic-like memory?. *J Neurosci*, *24*(8), 1948–1953. https://doi.org/10.1523/JNEUROSCI.2975-03.2004

Eichenbaum H. (2000). A cortical-hippocampal system for declarative memory. *Nat Rev Neurosci*, *1*(1), 41–50. https://doi.org/10.1038/35036213

Eichenbaum H. (2017). Prefrontal-hippocampal interactions in episodic memory. *Nat Rev Neurosci*, *18*(9), 547–558. https://doi.org/10.1038/nrn.2017.74

Eichenbaum, H., & Cohen, N. J. (2001). *From conditioning to conscious recollection: Memory systems of the brain. Oxford psychology series.* Oxford University Press.

Eichenbaum, H., Stewart, C., & Morris, R. G. (1990). Hippocampal representation in place learning. *J Neurosci*, *10*(11), 3531–3542. https://doi.org/10.1523/JNEUROSCI.10-11-03531.1990

Ekstrom, A. D., & Ranganath, C. (2018). Space, time, and episodic memory: The hippocampus is all over the cognitive map. *Hippocampus*, *28*(9), 680–687. https://doi.org/10.1002/hipo.22750

Ennaceur A. (2010). One-trial object recognition in rats and mice: methodological and theoretical issues. *Behav Brain Res*, *215*(2), 244–254. https://doi.org/10.1016/j.bbr.2009.12.036

Ennaceur, A., & Meliani, K. (1992). A new one-trial test for neurobiological studies of memory in rats. III. Spatial vs. non-spatial working memory. *Behav Brain Res*, *51*(1), 83–92. https://doi.org/10.1016/s0166-4328(05)80315-8

Farooq, U., & Dragoi, G. (2019). Emergence of preconfigured and plastic time-compressed sequences in early postnatal development. *Science*, *363*(6423), 168–173. https://doi.org/10.1126/science.aav0502

Feigley, D. A., Parsons, P. J., Hamilton, L. W., & Spear, N. E. (1972). Development of habituation to novel environments in the rat. *J Comp Physiol Psychol*, *79*(3), 443–452. https://doi.org/10.1037/h0032842

File S. E. (1978). The ontogeny of exploration in the rat: habituation and effects of handling. *Dev Psychobiol*, *11*(4), 321–328. https://doi.org/10.1002/dev.420110405

Freud, S. (1953). Three essays on the theory of sexuality. In J. Strachey (Ed.), The standard edition of the complete psychological works of Sigmund Freud (Vol. 7, pp. 135–243). London: Hogarth Press (original work published 1905).

Friedrich, M., Wilhelm, I., Born, J., & Friederici, A. D. (2015). Generalization of word meanings during infant sleep. *Nat Commun*, *6*, 6004. https://doi.org/10.1038/ncomms7004

Gómez, R. L., & Edgin, J. O. (2016). The extended trajectory of hippocampal development: Implications for early memory development and disorder. *Dev Cogn Neurosci*, *18*, 57–69. https://doi.org/10.1016/j.dcn.2015.08.009

Gómez, R. L., & Edgin, J. O. (2016). The extended trajectory of hippocampal development: Implications for early memory development and disorder. *Dev Cogn Neurosci*, *18*, 57–69. https://doi.org/10.1016/j.dcn.2015.08.009

Grieves, R. M., & Jeffery, K. J. (2017). The representation of space in the brain. *Behav Processes*, *135*, 113–131. https://doi.org/10.1016/j.beproc.2016.12.012

Guskjolen, A., Josselyn, S. A., & Frankland, P. W. (2017). Age-dependent changes in spatial memory retention and flexibility in mice. *Neurobiol Learn Mem*, *143*, 59–66. https://doi.org/10.1016/j.nlm.2016.12.006

Guskjolen, A., Kenney, J. W., de la Parra, J., Yeung, B. A., Josselyn, S. A., & Frankland, P. W. (2018). Recovery of "Lost" Infant Memories in Mice. *Curr Biol: CB*, *28*(14), 2283–2290.e3. https://doi.org/10.1016/j.cub.2018.05.059

Hafting, T., Fyhn, M., Molden, S., Moser, M. B., & Moser, E. I. (2005). Microstructure of a spatial map in the entorhinal cortex. *Nature*, *436*(7052), 801–806. https://doi.org/10.1038/nature03721

Haugland, K. G., Sugar, J., & Witter, M. P. (2019). Development and topographical organization of projections from the hippocampus and parahippocampus to the retrosplenial cortex. *Eur J Neurosci*, *50*(1), 1799–1819. https://doi.org/10.1111/ejn.14395

Hensch, T. K. (2005). Critical period plasticity in local cortical circuits. *Nat Rev Neurosci*, *6*(11), 877–888. https://doi.org/10.1038/nrn1787

Hofer, S. B., Mrsic-Flogel, T. D., Bonhoeffer, T., & Hübener, M. (2006). Lifelong learning: ocular dominance plasticity in mouse visual cortex. *Curr Opin Neurobiol*, *16*(4), 451–459. https://doi.org/10.1016/j.conb.2006.06.007

Houston-Price, C & Nakai S. (2004). Distinguishing novelty and familiarity effects in infant preference procedures. *Infant Child Dev*, 13,341–348, https://doi.org/10.1002/icd.364

Howe, M. L. (2019). Unravelling the nature of early (autobiographical) memory. *Memory, 27*(1), 115–121. https://doi.org/10.1080/09658211.2019.1537140

Huber, R., & Born, J. (2014). Sleep, synaptic connectivity, and hippocampal memory during early development. *Trends Cogn Sci*, *18*(3), 141–152. https://doi.org/10.1016/j.tics.2013.12.005

Hunter, M. A., & Ames, E. W. (1988). A multifactor model of infant preferences for novel and familiar stimuli. *Advances in Infancy Research, 5,* 69–95.

Inostroza, M., Binder, S., & Born, J. (2013). Sleep-dependency of episodic-like memory consolidation in rats. *Behav Brain Res*, *237*, 15–22. https://doi.org/10.1016/j.bbr.2012.09.011

Inostroza, M., Binder, S., & Born, J. (2013). Sleep-dependency of episodic-like memory consolidation in rats. *Behav Brain Res*, *237*, 15–22. https://doi.org/10.1016/j.bbr.2012.09.011

Jabès, A., Lavenex, P. B., Amaral, D. G., & Lavenex, P. (2011). Postnatal development of the hippocampal formation: a stereological study in macaque monkeys. *J Comp Neurol*, *519*(6), 1051–1070. https://doi.org/10.1002/cne.22549

Jablonski, S. A., Schreiber, W. B., Westbrook, S. R., Brennan, L. E., & Stanton, M. E. (2013). Determinants of novel object and location recognition during development. *Behav Brain Res*, *256*, 140–150. https://doi.org/10.1016/j.bbr.2013.07.055

Jin, W., Qin, H., Zhang, K., & Chen, X. (2020). *Adv Exp Med Biol*, *1284*, 63–90. https://doi.org/10.1007/978-981-15-7086-5_7

Jones, E. J., Pascalis, O., Eacott, M. J., & Herbert, J. S. (2011). Visual recognition memory across contexts. *Dev Sci*, *14*(1), 136–147. https://doi.org/10.1111/j.1467-7687.2010.00964.x

Josselyn, S. A., & Frankland, P. W. (2012). Infantile amnesia: a neurogenic hypothesis. *Learn Mem 19*(9), 423–433. https://doi.org/10.1101/lm.021311.110

Kaila K. (2011). A prestabilized harmony. *Neuron*, *71*(2), 201–202. https://doi.org/10.1016/j.neuron.2011.07.003

Kandel, E. R. (2001). The molecular biology of memory storage: a dialogue between genes and synapses. *Science*, 294(5544), 1030-1038. doi: 10.1126/science.1067020. PMID: 11691980

Kart-Teke, E., De Souza Silva, M. A., Huston, J. P., & Dere, E. (2006). Wistar rats show episodic-like memory for unique experiences. *Neurobiol Learn Mem*, *85*(2), 173–182. https://doi.org/10.1016/j.nlm.2005.10.002

Klinzing, J. G., Niethard, N., & Born, J. (2019). Mechanisms of systems memory consolidation during sleep. *Nat Neurosci*, *22*(10), 1598–1610. https://doi.org/10.1038/s41593-019-0467-3

Koski, J., Olson, I. R., & Newcombe, N. S. (2013). Tracking the eyes to see what children remember. *Memory*, *21*(3), 396–407. https://doi.org/10.1080/09658211.2012.735241

Kozareva, D. A., Cryan, J. F., & Nolan, Y. M. (2019). Born this way: Hippocampal neurogenesis across the lifespan. *Aging Cell*, *18*(5), e13007. https://doi.org/10.1111/acel.13007

Krüger, H. S., Brockmann, M. D., Salamon, J., Ittrich, H., & Hanganu-Opatz, I. L. (2012). Neonatal hippocampal lesion alters the functional maturation of the prefrontal cortex and the early cognitive development in pre-juvenile rats. *Neurobiol Learn Mem*, *97*(4), 470–481. https://doi.org/10.1016/j.nlm.2012.04.001

Kuhl, P. K. (2004). Early language acquisition: cracking the speech code. *Nat Rev Neurosci*, *5*(11), 831–843. https://doi.org/10.1038/nrn1533

Langston, R. F., & Wood, E. R. (2010). Associative recognition and the hippocampus: differential effects of hippocampal lesions on object-place, object-context and object-

place-context memory. *Hippocampus*, *20*(10), 1139–1153. https://doi.org/10.1002/hipo.20714

Langston, R. F., Ainge, J. A., Couey, J. J., Canto, C. B., Bjerknes, T. L., Witter, M. P., Moser, E. I., & Moser, M. B. (2010). Development of the spatial representation system in the rat. *Science (New York, N.Y.)*, *328*(5985), 1576–1580. https://doi.org/10.1126/science.1188210

Lavenex, P., & Banta Lavenex, P. (2013). Building hippocampal circuits to learn and remember: insights into the development of human memory. *Behav Brain Res*, *254*, 8–21. https://doi.org/10.1016/j.bbr.2013.02.007

Lavenex, P., Banta Lavenex, P., & Amaral, D. G. (2007). Postnatal development of the primate hippocampal formation. *Dev Neurosci*, *29*(1-2), 179–192. https://doi.org/10.1159/000096222

Loewen, I., Wallace, D. G., & Whishaw, I. Q. (2005). The development of spatial capacity in piloting and dead reckoning by infant rats: use of the huddle as a home base for spatial navigation. *Dev Psychobiol*, *46*(4), 350–361. https://doi.org/10.1002/dev.20063

Lorenz, K. (1935). Der Kumpan in der Umwelt des Vogels. Der Artgenosse als auslosendes Moment sozialer Verhaltensweisen. *J Ornithologie,* 83:137–215.

Lourenco, S. F., & Frick, A. (2014). *Remembering where: The origins and early development of spatial memory.* In P. J. Bauer & R. Fivush (Eds.), *The Wiley handbook on the development of children's memory* (p. 367–393). Wiley Blackwell.

Lupien, S. J., McEwen, B. S., Gunnar, M. R., & Heim, C. (2009). Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nat Rev Neurosci*, *10*(6), 434–445. https://doi.org/10.1038/nrn2639

Maguire, E. A., Burgess, N., & O'Keefe, J. (1999). Human spatial navigation: cognitive maps, sexual dimorphism, and neural substrates. C*urr Opin Neurobiol*, *9*(2), 171–177. https://doi.org/10.1016/s0959-4388(99)80023-3

Mendez, M., Arias, N., Uceda, S., & Arias, J. L. (2015). c-Fos expression correlates with performance on novel object and novel place recognition tests. *Brain Res Bull*, *117*, 16–23. https://doi.org/10.1016/j.brainresbull.2015.07.004

Mendez, M., Arias, N., Uceda, S., & Arias, J. L. (2015). c-Fos expression correlates with performance on novel object and novel place recognition tests. *Brain Res Bull*, 117, 16–23. https://doi.org/10.1016/j.brainresbull.2015.07.004

Morris, R. G., Garrud, P., Rawlins, J. N., & O'Keefe, J. (1982). Place navigation impaired in rats with hippocampal lesions. *Nature*, *297*(5868), 681–683. https://doi.org/10.1038/297681a0

Moser, E. I., Moser, M. B., & McNaughton, B. L. (2017). Spatial representation in the hippocampal formation: a history. *Nature neuroscience*, *20*(11), 1448–1464. https://doi.org/10.1038/nn.4653

Moser, M. B., Rowland, D. C., & Moser, E. I. (2015). Place cells, grid cells, and memory. *Cold Spring Harb Perspect Biol*, *7*(2), a021808. https://doi.org/10.1101/cshperspect.a021808

Muessig, L., Hauser, J., Wills, T. J., & Cacucci, F. (2015). A Developmental Switch in Place Cell Accuracy Coincides with Grid Cell Maturation. *Neuron*, *86*(5), 1167–1173. https://doi.org/10.1016/j.neuron.2015.05.011

Mullally, S. L., & Maguire, E. A. (2014). Learning to remember: the early ontogeny of episodic memory. *Dev Cogn Neurosci*, *9*, 12–29. https://doi.org/10.1016/j.dcn.2013.12.006

Nelson, K., & Fivush, R. (2004). The emergence of autobiographical memory: a social cultural developmental theory. *Psychol Rev*, *111*(2), 486–511. https://doi.org/10.1037/0033-295X.111.2.486

Newcombe N. S. (2019). Navigation and the developing brain. *J Exp Biol*, *222*(Pt Suppl 1), jeb186460. https://doi.org/10.1242/jeb.186460

Newcombe, N. S., Lloyd, M. E., & Ratliff, K. R. (2007). Development of episodic and autobiographical memory: a cognitive neuroscience perspective. *Adv Child Dev Behav*, 35, 37–85. https://doi.org/10.1016/b978-0-12-009735-7.50007-4

O'Keefe, J & Nadel, L. (1078). *The Hippocampus as a Cognitive Map*. Oxford University Press, New York.

O'Keefe, J., Burgess, N., Donnett, J. G., Jeffery, K. J., & Maguire, E. A. (1998). Place cells, navigational accuracy, and the human hippocampus. *Philos Trans R Soc Lond B Biol Sci*, *353*(1373), 1333–1340. https://doi.org/10.1098/rstb.1998.0287

Oyanedel, C. N., Durán, E., Niethard, N., Inostroza, M., & Born, J. (2020). Temporal associations between sleep slow oscillations, spindles and ripples. *Eur J Neurosci*, 10.1111/ejn.14906. Advance online publication. https://doi.org/10.1111/ejn.14906

Packard, M. G., & McGaugh, J. L. (1996). Inactivation of hippocampus or caudate nucleus with lidocaine differentially affects expression of place and response learning. *Neurobiol Learn Mem*, *65*(1), 65–72. https://doi.org/10.1006/nlme.1996.0007

Paul, C. M., Magda, G., & Abel, S. (2009). Spatial memory: Theoretical basis and comparative review on experimental methods in rodents. *Behav Brain Res*, *203*(2), 151–164. https://doi.org/10.1016/j.bbr.2009.05.022

Perone, S., & Spencer, J. P. (2013). Autonomous visual exploration creates developmental change in familiarity and novelty seeking behaviors. *Front Psychol*, *4*, 648. https://doi.org/10.3389/fpsyg.2013.00648

Perry, R., & Sullivan, R. M. (2014). Neurobiology of attachment to an abusive caregiver: short-term benefits and long-term costs. *Dev Psychobiol*, *56*(8), 1626–1634. https://doi.org/10.1002/dev.21219

Piaget, J. and Inhelder, B. (1956). The Child's Conception of Space. Routledge & Kegan Paul.

Ramsaran, A. I., Sanders, H. R., & Stanton, M. E. (2016). Determinants of object-in-context and object-place-context recognition in the developing rat. *Dev Psychobiol*, *58*(7), 883–895. https://doi.org/10.1002/dev.21432

Ramsaran, A. I., Schlichting, M. L., & Frankland, P. W. (2019). The ontogeny of memory persistence and specificity. *Dev Cogn Neurosci*, 36, 100591. https://doi.org/10.1016/j.dcn.2018.09.002

Ramsaran, A. I., Westbrook, S. R., & Stanton, M. E. (2016). Ontogeny of object-in-context recognition in the rat. *Behav Brain Res*, *298*(Pt A), 37–47. https://doi.org/10.1016/j.bbr.2015.04.011

Rasch, B., & Born, J. (2013). About sleep's role in memory. *Physiol Re*, *93*(2), 681–766. https://doi.org/10.1152/physrev.00032.2012

Ribordy Lambert, F., Lavenex, P., & Banta Lavenex, P. (2017). The "when" and the "where" of single-trial allocentric spatial memory performance in young children: Insights into the development of episodic memory. *Dev Psychobiol*, *59*(2), 185–196. https://doi.org/10.1002/dev.21479

Ribordy, F., Jabès, A., Banta Lavenex, P., & Lavenex, P. (2013). Development of allocentric spatial memory abilities in children from 18 months to 5 years of age. *Cogn Psychol*, *66*(1), 1–29. https://doi.org/10.1016/j.cogpsych.2012.08.001

Richmond, J. L., & Power, J. (2014). Age-related differences in memory expression during infancy: using eye-tracking to measure relational memory in 6- and 12-month-olds. *Dev Psychobiol*, *56*(6), 1341–1351. https://doi.org/10.1002/dev.21213

Roder, B. J., Bushneil, E. W., & Sasseville, A. M. (2000). Infants' Preferences for Familiarity and Novelty During the Course of Visual Processing. *Infancy*, *1*(4), 491–507. https://doi.org/10.1207/S15327078IN0104_9

Rose, S. A., Gottfried, A. W., Melloy-Carminar, P., & Bridger, W. H. (1982). Familiarity and

novelty preferences in infant recognition memory: Implications for information processing. *Dev. Psychol, 18*(5), 704–713. https://doi.org/10.1037/0012-1649.18.5.704

Rudy, J. W., Stadler-Morris, S., & Albert, P. (1987). Ontogeny of spatial navigation behaviors in the rat: dissociation of "proximal"- and "distal"-cue-based behaviors. *Behav Neurosci*, *101*(1), 62–73. https://doi.org/10.1037//0735-7044.101.1.62

Sanders, H. R., Heroux, N. A., & Stanton, M. E. (2020). Infant rats can acquire, but not retain contextual associations in object-in-context and contextual fear conditioning paradigms. *Dev Psychobiol*, *62*(8), 1158–1164. https://doi.org/10.1002/dev.21980

Sawangjit, A., Oyanedel, C. N., Niethard, N., Salazar, C., Born, J., & Inostroza, M. (2018). The hippocampus is crucial for forming non-hippocampal long-term memory during sleep. *Nature*, *564*(7734), 109–113. https://doi.org/10.1038/s41586-018-0716-8

Schiller, D., Eichenbaum, H., Buffalo, E. A., Davachi, L., Foster, D. J., Leutgeb, S., & Ranganath, C. (2015). Memory and Space: Towards an Understanding of the Cognitive Map. *J Neurosci*, *35*(41), 13904–13911.

Scott, R. C., Richard, G. R., Holmes, G. L., & Lenck-Santini, P. P. (2011). Maturational dynamics of hippocampal place cells in immature rats. *Hippocampus*, *21*(4), 347–353. https://doi.org/10.1002/hipo.20789

Sekeres, M. J., Winocur, G., & Moscovitch, M. (2018). The hippocampus and related neocortical structures in memory transformation. *Neurosci Lett*, *680*, 39–53. https://doi.org/10.1016/j.neulet.2018.05.006

Semple, B. D., Blomgren, K., Gimlin, K., Ferriero, D. M., & Noble-Haeusslein, L. J. (2013). Brain development in rodents and humans: Identifying benchmarks of maturation and vulnerability to injury across species. *Prog Neurobiol*, *106-107*, 1–16. https://doi.org/10.1016/j.pneurobio.2013.04.001

Sengupta P. (2013). The Laboratory Rat: Relating Its Age With Human's. *Int J Prev Med*, *4*(6), 624–630.

Shinskey, J. L., & Munakata, Y. (2005). Familiarity breeds searching: infants reverse their novelty preferences when reaching for hidden objects. *Psychol Sci*, *16*(8), 596–600. https://doi.org/10.1111/j.1467-9280.2005.01581.x

Shinskey, J. L., & Munakata, Y. (2010). Something old, something new: a developmental transition from familiarity to novelty preferences with hidden objects. *Dev Sci*, *13*(2), 378–384. https://doi.org/10.1111/j.1467-7687.2009.00899.x

Siegel, A. W., & White, S. H. (1975). The development of spatial representations of large-scale environments. *Adv Child Dev Behav*, *10*, 9–55. https://doi.org/10.1016/s0065-2407(08)60007-5

Squire L. R. (2004). Memory systems of the brain: a brief history and current perspective. *Neurobiol Learn Mem*, *82*(3), 171–177. https://doi.org/10.1016/j.nlm.2004.06.005

Tan, H. M., Bassett, J. P., O'Keefe, J., Cacucci, F., & Wills, T. J. (2015). The development of the head direction system before eye opening in the rat. *Curr Biol: CB*, *25*(4), 479–483. https://doi.org/10.1016/j.cub.2014.12.030

Tan, H. M., Wills, T. J., & Cacucci, F. (2017). The development of spatial and memory circuits in the rat. *Wiley Interdiscip Rev Cogn Sci*, *8*(3), 10.1002/wcs.1424. https://doi.org/10.1002/wcs.1424

Tang, A. C. (2001). Neonatal exposure to novel environment enhances hippocampal-dependent memory function during infancy and adulthood. *Learn Mem*, *8*(5), 257–264.

Travaglia, A., Bisaz, R., Sweet, E. S., Blitzer, R. D., & Alberini, C. M. (2016). Infantile amnesia reflects a developmental critical period for hippocampal learning. *Nat Neurosci*, *19*(9), 1225–1233. https://doi.org/10.1038/nn.4348

Travaglia, A., Steinmetz, A. B., Miranda, J. M., & Alberini, C. M. (2018). Mechanisms of critical period in the hippocampus underlie object location learning and memory in infant rats. *Learn Mem*, *25*(4), 176–182. https://doi.org/10.1101/lm.046946.117

Tse, D., Langston, R. F., Kakeyama, M., Bethus, I., Spooner, P. A., Wood, E. R., Witter, M. P., & Morris, R. G. (2007). Schemas and memory consolidation. *Science*, 316(5821), 76–82. https://doi.org/10.1126/science.1135935

Tse, D., Takeuchi, T., Kakeyama, M., Kajii, Y., Okuno, H., Tohyama, C., Bito, H., & Morris, R. G. (2011). Schema-dependent gene activation and memory encoding in neocortex. *Science*, 333(6044), 891–895. https://doi.org/10.1126/science.1205274

Tsujimoto S. (2008). The prefrontal cortex: functional neural development during early childhood. *Neuroscientist*, *14*(4), 345–358. https://doi.org/10.1177/1073858408316002

Tulving, E. (1993). What Is Episodic Memory?. *Current Directions in Psychological Science*, *2*(3), 67–70. https://doi.org/10.1111/1467-8721.ep10770899

Tzakis, N., & Holahan, M. R. (2020). Investigation of GluA1 and GluA2 AMPA receptor subtype distribution in the hippocampus and anterior cingulate cortex of Long Evans rats during development. *IBRO Rep*, *8*, 91–100. https://doi.org/10.1016/j.ibror.2020.03.003

Vorhees, C. V., & Williams, M. T. (2014). Assessing spatial learning and memory in rodents. *ILAR J*, *55*(2), 310–332. https://doi.org/10.1093/ilar/ilu013

Wagner, S. H., & Sakovits, L. J. (1986). A process analysis of infant visual and cross-modal recognition memory: Implications for an amodal code. *Advances in Infancy Research, 4,* 195–217, 240–245

Watson, R. E., Desesso, J. M., Hurtt, M. E., & Cappon, G. D. (2006). Postnatal growth and morphological development of the brain: a species comparison. *Birth defects research. Part B, Birth Defects Res B Dev Reprod Toxicol*, *77*(5), 471–484. https://doi.org/10.1002/bdrb.20090

Westbrook, S. R., Brennan, L. E., & Stanton, M. E. (2014). Ontogeny of object versus location recognition in the rat: acquisition and retention effects. *Dev Psychobiol*, *56*(7), 1492–1506. https://doi.org/10.1002/dev.21232

Whishaw, I. Q., & Mittleman, G. (1986). Visits to starts, routes, and places by rats (Rattus norvegicus) in swimming pool navigation tasks. *J Comp Psychol*, *100*(4), 422–431.

White, N. M., & McDonald, R. J. (2002). Multiple parallel memory systems in the brain of the rat. *Neurobiol Learn Mem*, *77*(2), 125–184. https://doi.org/10.1006/nlme.2001.4008

Wills, T. J., & Cacucci, F. (2014). The development of the hippocampal neural representation of space. *Curr Opin Neurobiol*, *24*(1), 111–119. https://doi.org/10.1016/j.conb.2013.09.006

Wills, T. J., Cacucci, F., Burgess, N., & O'Keefe, J. (2010). Development of the hippocampal cognitive map in preweanling rats. *Science (New York, N.Y.)*, *328*(5985), 1573–1576. https://doi.org/10.1126/science.1188224

Wills, T. J., Muessig, L., & Cacucci, F. (2013). The development of spatial behaviour and the hippocampal neural representation of space. *Philos Trans R Soc Lond B Biol Sci*, *369*(1635), 20130409. https://doi.org/10.1098/rstb.2013.0409

Winocur, G., & Moscovitch, M. (2011). Memory transformation and systems consolidation. *J Int Neuropsychol Soc*, *17*(5), 766–780. https://doi.org/10.1017/S1355617711000683

Zeanah, C. H., Egger, H. L., Smyke, A. T., Nelson, C. A., Fox, N. A., Marshall, P. J., & Guthrie, D. (2009). Institutional rearing and psychiatric disorders in Romanian preschool children. *Am J Psychiatry*, *166*(7), 777–785. https://doi.org/10.1176/appi.ajp.2009.08091438

List of papers and statement of contributions

Study 1

Contreras, M. P., Born, J., & Inostroza, M. (2019). The expression of allocentric object-place recognition memory during development. *Behav Brain Res*, 372, 112013. https://doi.org/10.1016/j.bbr.2019.112013

I, Born, J. and Inostroza, M. designed the experiments. I performed the experiments and collected the data. I analyzed the data with inputs from Born, J. and Inostroza, M. I wrote the manuscript. Born, J. and Inostroza, M. edited the manuscript.

Study 2

Xia, S., Contreras, M. P., Mendez, M., Born, J. and Inostroza, M. (*under revision*). Unfolding of spatial representation at system level in infant rats.

I, Xia, S., Born, J. and Inostroza, M. designed the experiments. Xia, S. performed the experiments and collected the behavioral data. Mendez, M. collected the c-fos data. Xia, S. analyzed the data with inputs from me, Jan born and Mario inostroza. Xia, S., Born, J. and Inostroza, M. wrote the manuscript.

Study 3

Contreras, M. P., Mendez, M., Born, J. & Inostroza, M. (*under revision*). Discrete spatial experience during infancy builds schema memory for adult learning.

I, Born, J. and Inostroza, M. designed the experiments. I performed the experiments and collected the behavioral the data. Mendez, M. collected the c-fos data. I and Inostroza M. analyzed the data with input from Born, J. Inostroza and Born, J. wrote the manuscript and conceived the study.

Appended Papers

Study 1 – The expression of allocentric object-place recognition memory during development.

Behavioural Brain Research 372 (2019) 112013

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Research report

The expression of allocentric object-place recognition memory during development

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ABSTRACT

The allocentric representation of space is a fundamental pillar of episodic experience. In infant rats, the neural circuitry underlying the formation of allocentric spatial representations is functioning from early on when eyes are opening, i.e., before postnatal day (PD) 15. However, it remains unclear when and how during early development rats use these representations to regulate spatial behavior. Here, we studied indicators of memory-based spatial navigation using a classical object-place recognition (OPR) task set-up in infant (PD15), pre-weanling (PD18), juvenile (PD25), peri-adolescent (PD31), adolescent (PD38, PD48), and young adult rats (PD84). On the task, rats explored an arena with two identical objects, and memory was tested in a recall phase 3 h later in the same arena with, one object displaced from its original location. Only at adolescence (PD38), rats showed the typical adult-like expression of allocentric spatial memory with a preferential exploration of the object at the novel location. However, the first expression of allocentric spatial memory was revealed already in PD18 rats, which contrasting with PD84 rats, showed a preference to explore the object at the familiar location. At PD31, rats showed a null preference between the object-locations. Nevertheless, spatial memory at this age expressed in a preference for the zone including the familiar object-location. In PD15 rats, we found no evidence for a memory-based organization of spatial behavior. In conclusion, although rats might be able to form allocentric neuronal representations of space already earlier, only from PD18 on, such representations are used to organize spatial behavior, with a motivational shift from familiarity to novelty-driven navigation occurring during adolescence.

1. Introduction

The ability to form representations of spatial relationships among elements of the environment is critical for navigation and, generally, for the regulation of adaptive behavior through episodic experience. Spatial representations can be egocentric, i.e., defining spatial elements in relation to the own body, or allocentric. Allocentric representations map the spatial relationships among environmental elements independently of the own body position, and thus allow for more flexible navigation based on distal cues [1]. During early life, allocentric representations may develop as an advanced form of egocentric spatial representations [2]. However, more recent accounts propose a parallel development of both representational systems, although the development of allocentric mapping is thought to be slower and possibly more experience-driven [3,4].

The formation of allocentric spatial representations relies on a network essentially comprising hippocampal place cells, entorhinal grid cells and more distributed head direction cells, the firing of which is modulated by the animal's position and orientation in space [5]. Recordings of neural activity provided consistent evidence that the different neural components of this spatial network are functioning already early during development even before extensive experience, i.e., in infantile and pre-weanling rats before PD15 [3,6–8]. Paradoxically, it is unclear whether animals can actually use this system for a memory-based navigation in space at this early stage. Previous studies using the hidden platform version of the Morris water maze (MWM) task indicated that allocentric spatial navigation begins to emerge only after PD20 in rats [9–12]. Compared to rats, mice exhibit an earlier spatial

Abbreviations: OPR, object-place recognition; PD, postnatal day; MWM, Morris water maze; ODI, object discrimination index; OZDI, object zone discrimination index

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memory development. Using the MWM task, mice were able to find the hidden platform at the age of PD15 [13]. In the object-place recognition (OPR) task, short-lived spatial representations (kept over ≤10 min) appeared to be functional in rats at PD16. The OPR tasks differ considerably from the MWM, particularly in that it does not involve negative reinforcement and is, hence, less stressful, and in that it, unlike the MWM task, relies on only a single acquisition trial. However, both tasks are hippocampal-dependent and seem to emerge closely in ontogeny. In the OPR task more persistent representations, maintained over more than 2 h, were revealed only after PD24 [14–16]. Importantly, in all these studies, visual cues were provided within the arena and on the walls of the room, making it difficult to disentangle whether the animals used an egocentric or allocentric strategy to perform the task [17,18]. To address this issue, here, we adopted the allocentric version of an OPR task-set up to examine the ontogeny of spatial memory in rats between PD15 and adulthood.

The OPR task requires hippocampal function and, in adult rodents, is used to assess spatial memory based on the animal´s natural tendency to preferentially explore novel stimuli and places over familiar ones [19–22]. During a single encoding phase, the animal explores an arena containing two identical objects. At the later recall test session in the same arena, one of the objects is moved to a novel location, and the preferential exploration of the object at the novel location, compared to the object at the familiar location, is taken to measure spatial memory. Whereas, in adults, this behavioral pattern is well documented [23], the use of the OPR task in infant rats has to take into account that the motivational preference for novelty versus familiarity might change during development [24,25]. In the wild, infant rats within the first weeks of life begin to leave the home nest to forage for food. This signals an important shift from being attracted to the familiar/known nest to the preferential exploration of novel places that might likewise affect the animal's behavioral preference (for either the familiar or novel object-location) in the OPR task. Using the OPR task for the assessment of spatial abilities in infant rats, it should be considered that the task requires the animal to readily discriminate the objects from the surrounding arena, because the ability to distinguish objects from their background might have a different developmental trajectory [15,26,27].

Against this backdrop, in the present study we used the original version of the OPR task set-up but introduced additional behavioral measures, in order to achieve an unbiased assessment of the presence of persisting (> 3 h) allocentric spatial representations in our youngest rat groups. Moreover, different from previous approaches, we took into account a possible exploration preference for the familiar (rather than novel) object location. We found navigation based on allocentric representations to occur not until PD18, with a motivational shift from preferential orientation towards familiar zones and objects to preferential orientation towards novel objects at early adolescence.

2. Material and methods

2.1. Animals

Ninety-nine male Long-Evans rats were used for the experiments. Rats were grouped according to the postnatal day (PD) the OPR task was performed (Fig. 1A), i.e. the groups of infantile (PD15), preweanling (PD18), juvenile (PD25), peri-adolescent (PD31), adolescent (PD38, PD48), and of young adult (PD84) rats. Each group included 12 rats except the PD18 group (n = 21) and PD25 group (n = 18). An additional group of PD15 pups (n = 13) was tested in the OPR task without prior habituation to the spatial arena (PD15-NoHab group, see below). All rats of the PD15 groups had opened their eyes and had already started to explore their home cage surroundings on the day of the experiment (as indicated by qualitative observations made during handling). Each group derived from two litters of 6 pups (except the PD18 which derived from 4 litters, one of those with only 3 pups and PD25 derived from 3 litters). In total 19 litters were used for the whole experiment. Four litters were born in our own animal facilities (each litter was culled to 6 pups one or two days after parturition). The remaining pups arrived (from Janvier, Le Genest-Saint-Isle, France) in our facilities at least 3 days before any manipulation in order to allow acclimatization. The pups were maintained with their mother until the weaning (PD21). The animal colony was kept at a room temperature of 22 ± 1 °C, on a 12 h/12 h light/dark cycle (lights on at 6:00 h). All rats had free access to food and water throughout the experiments. All experimental procedures were performed in accordance with the European animal protection laws (Directive 2010/63/EU) and were approved by the Baden-Württemberg state authority.

2.2. Experimental procedures and task

All procedures were performed between 7:00–14:00 h (i.e., the light phase). Animals were first handled for 5 min on 5 consecutive days. For the PD15, PD18 and PD25 groups, the handling procedures included the mothers in order to diminish potential stress caused by the manipulations of the nest. On each of the following 3 days, a 10-min habituation session was performed in all groups, except in the PD15-NoHab group, which did not undergo habituation to keep spatial experience before testing at a minimum. In the habituation sessions, the rats were allowed to freely explore the empty open field. In each session, they were introduced into the arena from a different side. After the session, the rats were, in pairs, habituated to the rest-box for 6 h, except the pre-weanling PD15 and PD18 pups which returned to their mothers in the home cage.

On the day after the last habituation session, the rats were tested on the OPR task (Fig. 1B). Two copies of the same object were placed 9 cm and 15 cm distant from the walls of the respective smaller and larger open field (see section 2.3). The encoding phase comprised a 5-min interval during which the rat could explore the two objects. Thereafter during the 3 -hs retention interval, pairs of rats were left undisturbed in the rest-box. Rats of the PD15 and PD18 groups, instead, were returned to the home cage. During the subsequent recall phase, one of the objects of the encoding phase was placed at a novel location while the other object remained at the same (familiar) location as in the encoding phase. The recall phase comprised another 5-min interval during which the animal could explore the objects. Importantly, each rat was introduced into the arena from a different side in the encoding phase and in the recall phase, with the rat always faced the respective wall of the arena in order to prevent that the entrance position was used as a proximal cue [17,28]. Object locations during the recall phase were counterbalanced across rats.

2.3. Apparatus and objects

The OPR task was performed in a quadratic dark grey open field. Two different sizes of the arena were used depending on the rats' age. The PD15, PD18, PD25, and PD31 groups performed the task in a smaller arena (43 cm x 43 cm with 35 cm high walls) and the PD38, PD48 and PD84 groups in a larger arena (77 cm x 77 cm with 37 cm high walls). Objects were glass bottles of different shapes, filled with water or sand of different colors. They had sufficient weight to ensure the rats could not displace them. Two different sets of objects were used depending on the age. For the PD15, PD18, PD25 and PD31 groups the height of the objects was 10–18 cm, and for the PD38, PD48 and PD84 groups was 22–29 cm.

To facilitate allocentric navigation, a number of distal cues were available. The North side of the arena was headed towards a white wall whereas the East and West sides were surrounded by a grey curtain. The South side of the arena faced a removable black curtain (which was used as entrance for the experimenter). Additional discrete distal cues were provided on the ceiling: a brown wood square (40 cm x 40 cm) located 120 cm above the open field and 36 cm below the ceiling. In the

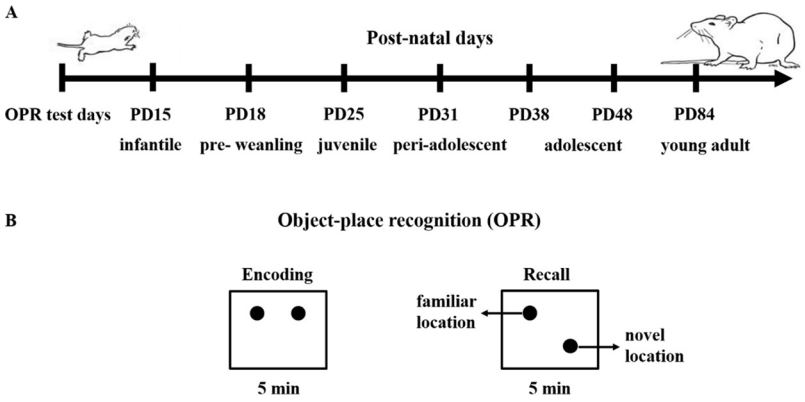

Fig. 1. A Experimental design. Seven groups of rats were tested on an Object-Place Recognition (OPR) task at different postnatal days (PD) representing different developmental stages (infantile: PD15, pre-weanling: PD18; juvenile: PD25, peri-adolescent: PD31; adolescence: PD38, PD48, and young adult: PD84). **B** OPR task set up. The rat explored an arena with two identical objects for 5 min, once during the Encoding phase and again during the Recall phase, with the two phases separated by a 3 -h retention interval. In the recall phase, one of the objects was displaced to a novel location while the other remained in the same familiar location as during the encoding phase. Each rat was placed into the arena close to and facing one of the walls, with different entry-walls used for an individual rat during encoding and recall phases. In the retention interval, the rat was placed to a separate rest-box for 3 h, except PD15 and PD18 rats which were returned to the mother in the home cage.

center of the square was a metal square (5 cm x 7 cm) which simultaneously served as holder for the video camera. At two sides, a pink ball (10 cm diameter) and a light-brown cartoon box (25 cm x 25 cm x 10 cm), respectively, were attached to the curtains. Two fluorescent strip lights placed on the floor of the room provided indirect light. White noise was presented at a constant intensity during all procedures, to mask any disturbing sounds. Objects and the open field were cleaned thoroughly between trials with 70% ethanol solution. The rest box used in the retention interval measured 35 cm x 35 cm, with 45 cm high walls.

2.4. Data collection and analysis

The rat's behavior was video-recorded during the encoding and recall phase and visually scored offline by an experienced experimenter using the ANY-Maze tracking software (Stoelting Europe, Dublin, Ireland). Exploration was defined by the rat directing its nose to the object and sniffing. Climbing on an object or sitting next to it without any signs of active exploration was not included.

Allocentric spatial memory was analysed using object related and non-object related spatial measures. As an object related measure we calculated the object discrimination index (ODI) which represents the standard way to assess OPR memory in adult rats, and is defined by the formula: ODI = [(exploration time for novel object-location – exploration time for familiar object-location) / (exploration time for novel object-location + exploration time for familiar object-location)]. Note, the ODI yields meaningful data only if the animal exhibits minimum amounts of exploration of the two objects. Therefore, only rats that had explored each of the two objects for at least 1 s during the encoding phase were included in the analyses. Based on this criterion (and the removal of statistical outliers, see section 2.5), the final group size for the analyses of the ODI was for PD15 n = 0, PD18 n = 15, PD25 n = 15, PD31 n = 10, PD38 n = 10, PD48 n = 11, and PD84 n = 12. As a complementary object related measure of spatial memory, we calculated an "object-zone discrimination index" (OZDI). For this purpose, the open field was divided into 4 identical quadrants. Then, the following formula was applied: OZDI = [(time in novel object-location zone) – (time in familiar object-location zone) / (time in novel object-location zone) + (time in familiar object-location zone)], with the zones defined by the quadrant of the arena in which the respective object was located (Fig. 4A).

In addition, the total exploration time across both objects, the proportion of total exploration time spent with the novel object-location, and the proportion of total exploration time spent with the familiar object-location were calculated for each animal. Analogue measures were calculated using the total time the animal spent in both novel-location and familiar-location object zones. The total distance travelled, the object latency and the total time of object exploration (cumulative and non-cumulative) during the encoding and recall phase, respectively, were used as indicators of nonspecific activation and exploration motivation. As a behavioural indicator of anxiety, we calculated the percent time of the 5-min recall interval spent in the central zone.

As non-object related measure of spatial memory, we analysed the time an individual animal spent in its "familiar zone". This zone was defined by the quadrant of the arena in which the animal spent most of the time during the encoding phase. A preference for the familiar zone during the recall phase was then analysed by comparing the time spent in the familiar zone with the average time spent in the three other zones during the recall (Fig. 5A).

2.5. Statistical analyses

All statistical analyses were performed using SPSS software (IBM, Armonk, NY, USA). Generally, statistical outliers were excluded from the analyses when the respective value exceeded ± 2 standard deviations from the group´s mean. Analyses of ODI, OZDI and related behavioral measures concentrated on cumulative scores for the first 1 min and the first 3 min of the recall phase. The first 3-min interval of exploration was consistently found to most sensitively reflect behavioral memory effects on the task in adult rats (e.g., Chambon et al., 2011; Ozawa et al., 2011 [29,30]). This was confirmed in a supplementary analysis of the present data showing that total object exploration time in all age groups decreased over the 5-min recall phase and that all groups showed, on average, maximum total exploration time within the first 3 min (Figure S2 D). To analyse age effects, we used analyses of variance (ANOVA) with Age as group factor and Time interval during the recall phase (1-min and 3-min) as repeated measures factor. To separately assess in sub-ANOVA the exploration times for the novel-location and familiar-location objects, an additional repeated measures factor Object location was introduced. Analyses of the time spent in the "familiar zone" comprised an additional factor Zone to account for differences in time spent in this versus the other 3 zones. Time spent in the familiar zone was also analysed using chi^2 test. Significant ANOVA main or interaction effects were followed by post-hoc pairwise *t*-tests. One sample *t*-test was used to test whether ODI and OZDI values differed from chance level (zero) whereby in the text the interval (1-min vs. 3-min) is reported showing the most robust significance. Estimates of effect size, i.e., Cohen's *d* was also provided for the significant terms. Regression analyses were applied to characterize developmental trajectories across the age groups. All results are reported as means ± SEM. A $p < 0.05$ was considered significant.

3. Results

3.1. Object discrimination index

Our first approach to assess allocentric spatial memory on the OPR task during the recall phase was based on the classical object place discrimination index (ODI), i.e., the proportion of time the animal spent

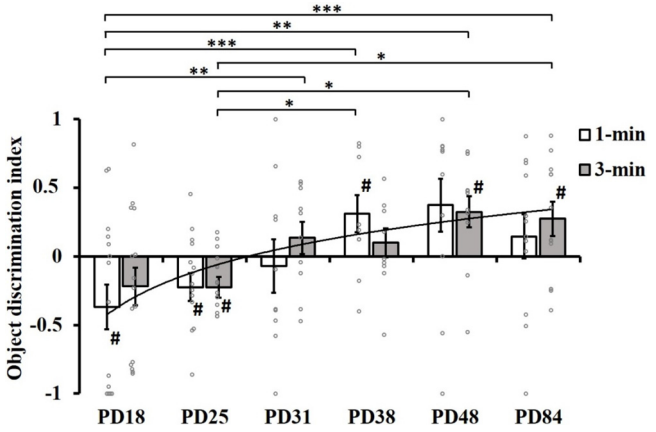

Fig. 2. Mean (± SEM) object discrimination index (ODI) during recall phase for each of 6 age groups (Data distribution overlaid). Positive ODI indicates preferential exploration of object at novel location; negative ODI indicates preferential exploration of object at familiar location. ODIs are shown for the first 1-min (white bars) and 3-min intervals (grey bars) of the recall phase. # $p < 0.05$ for one-sample *t*-test against chance level, i.e. zero. Note, groups PD18 and PD25 showed significant preference for the object at the familiar location whereas groups PD38, PD48, and PD84 showed preference for the object at the novel location. Group PD31 showed null preference. Brackets on top indicate differences between age groups: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, for LSD post-hoc tests. Logarithmic regression line across age groups is overlaid ($y = 0.3832\ln(x) - 0.3538$, $p < 0.001$). For regression analysis the time interval (1 vs. 3 min) with the greater p-value (against zero) was used. Note, PD15 rats were excluded from this analysis because they did not show sufficient object exploration.

exploring the object at the novel location relative to the exploration time spent with the object at the familiar location. Accordingly, a positive ODI indicates an exploration preference for novelty whereas a negative ODI indicates preference for familiarity. Because the ODI yields valid data only if the animal exhibits minimum amounts of exploration, only rats that explored each object for at least 1 s during the encoding phase were included in the analyses (see Material and methods). Notably, none of the pups tested at PD15 reached this criterion, indicating that at this very early age, object exploration cannot be used to validly assess spatial memory. In fact, discrimination scores calculated for this group without this criterion impressed by their huge variability (0.33 ± 0.66 and -0.11 ± 0.40 discrimination ratios at PD15 for 1-min and 3-min, respectively).

Consequently, the following analyses were restricted to the remaining age groups tested at PD18, PD25, PD31, PD38, PD48 and at the young adult age of PD84. The ODI during the recall phase systematically differed across Age groups ($F(5, 58) = 3.812$, $p = 0.005$, for main effect of Age, Fig. 2), regardless of the Time interval ($F(1, 67) = 0.439$, $p = 0.510$, for main effect of Time interval, $F(5, 67) = 1.172$, $p = 0.332$, for Age x Time interval). Interestingly, pre-weanling PD18 and juvenile PD25 groups displayed a significant negative ODI, i.e., rats showed a preferential exploration of the object at the familiar location after the 3 -h retention interval (PD18 1-min: $t(14) = -2.244$, $p = 0.042$, $d = -0.578$, and PD25 1-min: $t(14) = -2.223$, $p = 0.043$, $d = -0.574$, 3-min: $t(14) = -2.946$, $p = 0.011$, $d = -0.761$ respectively for post-hoc *t*-test against zero, Fig. 2). The peri-adolescent PD31 group showed a null preference, i.e., although both objects were explored during the encoding phase, no preference was expressed during the recall phase ($p > 0.05$ for t-tests against zero level). While the adolescent (PD38 and PD48) as well as the young adult rats (PD84) showed a significant positive discrimination index, indicating a preference for the object at the novel location (PD38 1-min: $t(9) = 2.312$, $p = 0.046$, $d = 0.731$; PD48 3-min: $t(10) = 1.938$, $p = 0.017$, $d = 0.584$; PD84 3-min: $t(11) = 2.215$, $p = 0.049$, $d = 0.639$). There were no differences between the PD38, PD48 and PD84 groups ($p > 0.05$), suggesting that the novelty preference is fully established during adolescence. The gradual shift from preference for the familiar object-location to the novel object-location from early life to adulthood could be described by a logarithmic regression function which explained 20% of the variance in the ODI across Age groups and levelled out at about PD38 ($F(1, 71) = 17.218$, $p < 0.001$, Fig. 2).

ODI values in Fig. 2 suggested that the youngest rats (PD18) expressed their preference for the familiar object-location in particular during the first minute of the recall phase whereas the preference for the novel object-location in adult rats (PD84) was expressed most robustly over the 3-min interval. However, in an additional analysis comparing the youngest (PD18) and oldest (PD84) rat groups, the Time interval factor did not interact with the proportion of time spent with either the familiar or novel object-location in the two age groups ($F(1, 22) = 0.003$, $p = 0.953$; for Object location x Time interval x Age) excluding a robust temporal change across age in the expression of respective location preferences. The analysis confirmed the shift from preference for the familiar object-location at PD18 to the novel object-location at PD84 ($F(1, 22) = 5.714$, $p = 0.026$, for Object location x Age).

Overall, these data support the conclusion that already pre-weanling rats at PD18 are capable of navigating based on allocentric spatial mapping of objects encountered 3 h before. It is merely the behavioral expression of the memory that systematically changes during development: Whereas pre-weanling and juvenile rats use the object-place memory to preferentially explore the familiar object-location, peri-adolescent rats express a null preference, and from adolescence on, rats show the adult-like preference for the novel object-location.

In a control study (n = 8) we confirmed that pre-weanling rats (at PD18) likewise expressed a preference for the familiar object-location when the retention interval between encoding and recall testing was shortened to only 5 min. Unexpectedly, in this study robust significance was only reached at the firsts 4-min and 5-min time intervals of the recall phase (all $p < 0.05$ for t-tests against zero, Figure S1) and not at the firsts 1-min or 3-min intervals, as the group of the main experiment subjected to a 3-hour retention interval.

3.2. Total exploration time, object latency, and distance travelled

In order to examine non-specific developmental trends in locomotion and object exploration we analysed the total time the rats spent to explore each object, the time spent to approach any of the two objects for the first time (latency of first approach), and the total distance travelled during the encoding and recall phase. At encoding, all 3 parameters differed among the groups ($F(6, 92) = 7.576$, 23.668, and 31.591, respectively, $p < 0.001$). Post hoc t-tests revealed parallel age patterns, i.e., the PD15 rats showed distinctly less total exploration time across both objects, a significantly longer approach latency, and a distinctly shorter distance travelled during the encoding phase than the older age groups (see Fig. 3 for statistical significances). Moreover, the total object exploration time was significantly longer in the adult PD84 rats than in all other groups. Similar results were found during the recall phase (Figure S2). Altogether, this picture suggests that object exploration capability is not sufficiently mature at PD15.

3.3. Object-location zone preference

Since young rats, particularly the PD15 group did not show full-blown exploration of the objects, we investigated whether a discrimination index based on the rat's tendency to spend relatively more time in the zone (quadrant) of the arena with either the familiar or novel object-location might be more sensitive to reflect object-location memory than on actual object exploration (see Material and methods). Analyses of the respective object-zone discrimination index (OZDI) were restricted to the four youngest age groups (PD15, PD18, PD25, PD31), and indicated an overall significant negative OZDI, i.e.,

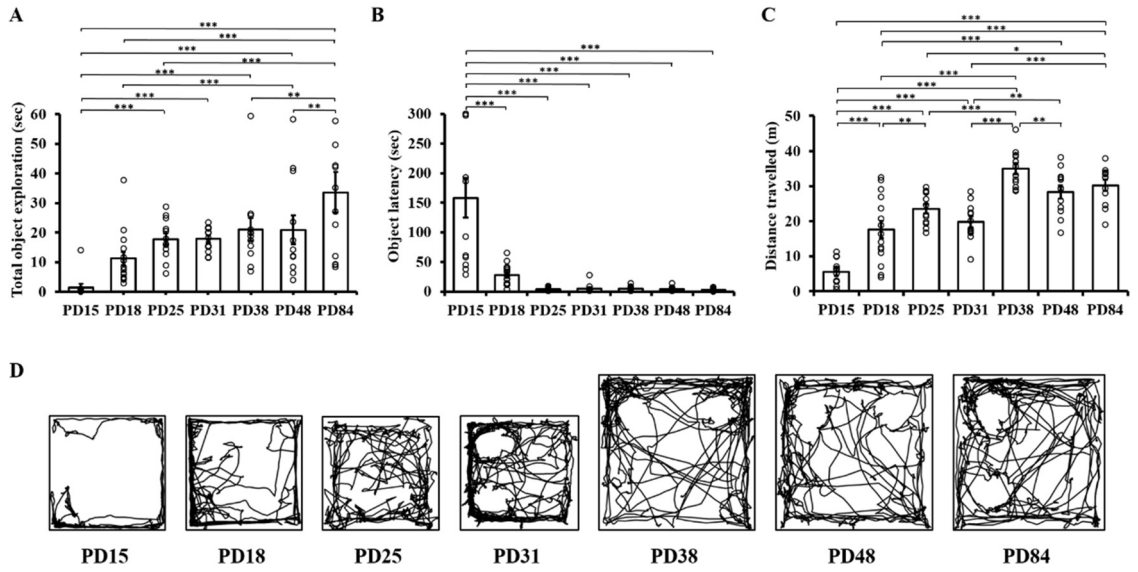

Fig. 3. Means (± SEM) for **A** total object exploration time, **B** latency of first approach, and **C** distance travelled during the 5-min encoding phase for the different age groups. (Data distribution overlaid) Brackets on top indicate differences between age groups: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, for LSD post-hoc tests. **D** Representative diagrams of individual path travelled in the open field for each group.

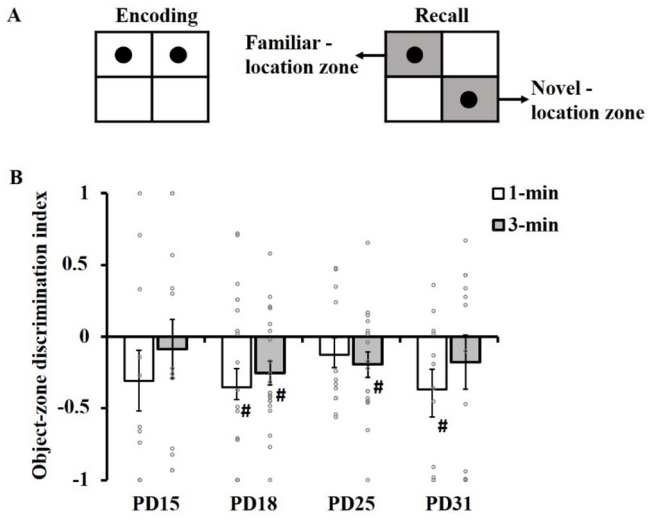

Fig. 4. A Object-zone discrimination index (OZDI) as a complementary object-related measure of spatial memory. A negative OZDI indicates that the rat during the recall phase spent more time in the quadrant of the arena containing the familiar object-location (familiar-location zone) than in the quadrant containing the novel object-location (novel-location zone), and vice versa, a positive OZDI indicates more time spent in the quadrant with the novel object-location. **B** Mean (± SEM) OZDI during recall phase for the four younger age groups (Data distribution overlaid). OZDIs are shown for the first 1-min (white bars) and 3-min intervals (grey bars) of the recall phase. # $p < 0.05$, for one-sample *t*-test against chance level, i.e., zero. Note, groups PD18, PD25 and PD31 showed significant negative OZDIs, There were no significant differences between age groups. Note highly variable OZDI in PD15 rats.

relatively enhanced time spent in the zone that contained the familiar object-location compared to the zone that contained the novel object-location ($t(61) = -4.575$, $p < 0.001$, $d = -0.581$), with no differences among Age groups ($F(3, 61) = 0.320$, $p = 0.811$). Separate analyses for each group revealed a significant negative OZDI above chance level for the pre-weanling PD18 group, the juvenile PD25 group and for the peri-adolescent PD31 group (PD18 1-min: $t(20) = -2.668$, $p = 0.015$, $d = -0.581$, 3-min: $t(20) = -3.014$, $p = 0.007$, $d = -0.657$, PD25 3-min: $t(17) = -2.324$, $p = 0.033$, $d = -0.548$, and PD31 1-min: $t(11) = -2.571$, $p = 0.026$, $d = -0.742$, Fig. 4). Thus, pre-weanling (PD18) and juvenile rats (PD25) expressed a surprisingly consistent familiarity preference in both object (ODI) and zone location indexes (OZDI), whereas peri-adolescent rats (PD31) exhibited a null preference in the ODI, but a familiarity preference in the OZDI, suggesting the OZDI to be the more sensitive measure of spatial memory at this age.

3.4. Familiar zone preference

To assess whether PD15 rats might form spatial representations independent of their capabilities to distinguish and explore objects, we analysed the mere preference for a certain zone (quadrant) in the arena. The “familiar zone” was defined by quadrant of the arena where the rat spent most of the time during the encoding phase. For the recall phase, we compared the time the animal spent in this familiar zone with the average time spent in the three other zones (other zones). Note that the classification of the zones was completely independent of the locations of the objects, i.e., the zone could or could not contain an object (Fig. 5A, B). In these analyses, an additional group of PD15 rats was included (PD15-NoHab, n = 13) which did not undergo prior habituation sessions and, thus obtained only a minimum of spatial experience prior to the experimental testings.

The familiar zone differed among the rats and did not coincide with the rat´s entrance side to the arena. A two-way ANOVA including Age and Zone (familiar vs other zones) as factors on the recall phase revealed no main effect of Age ($F(7, 92) = 0.900$, $p = 0.510$), but a main effect of Zone ($F(1, 92) = 5.677$, $p < 0.019$) without significant Age x Zone interaction effect ($F(7, 92) = 0.915$, $p < 0.498$). Post hoc pairwise comparisons showed no significant preferences for the “familiar zone” at any postnatal day (all $p > 0.06$, Fig. 5C). Six of the 12 pups of the PD15 group and 4 of the 13 pups of the PD15-NoHab group spent most of the time during the recall phase in the familiar zone, with this distribution failing to reach significance ($chi^2 = 4.667$, $p = 0.198$ and chi^2 4.538, $p = 0.209$), also when both groups were combined ($chi^2 = 6.840$, $p = 0.077$). Also, the proportion of time the PD15 rats spent in the “familiar zone” during the encoding phase did not correlate with that during the recall phase ($r = -0.298$ and 0.001, $p > 0.34$, for PD15 and PD15-NoHab groups, respectively). Altogether, these results suggest that at PD15, rats do not rely on persistent allocentric representations to organize spatial behavior.

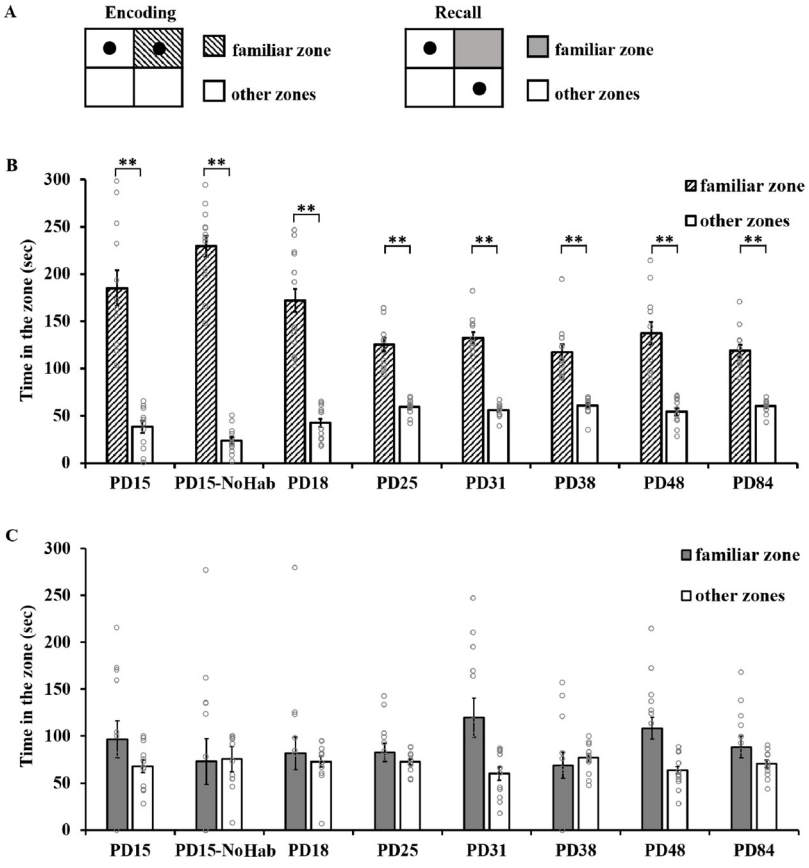

Fig. 5. Preference for the "familiar zone" as a non-object related measure of spatial memory. **A** The familiar zone was defined by the quadrant of the arena in which the individual rat spent most of the time during the encoding phase (in the illustrated case: hatched area), regardless of the locations of the objects. The "familiar zone" preference during the recall phase was determined by the time the rat spent in the familiar zone in comparison with the average time spent in the three remaining quadrants during this phase. **B** Mean (± SEM) time the animal spent in the most explored quadrant during the encoding phase (hatched bars) vs average time spent in the other three quadrants (empty bars), separately for each age group (Data distribution overlaid). All groups showed a significant preference for one quadrant during encoding, i.e., the familiar zone (*** $p <$ 0.001, for paired *t*-test). Note, the familiar zone differed across animals and did not coincide with the side the rat entered the arena. **C** Mean (± SEM) time in "familiar zone" (grey bars) and for the average time in the 3 remaining zones (empty bars) during recall phase, separately for the different age groups (Data distribution overlaid). None of the groups showed a significant preference for the familiar zone over the other zones.

4. Discussion

We examined capabilities to form persistent allocentric spatial representations during early life in rats using the object-place recognition (OPR) task. We found that such representations keeping over a 3 -h retention interval can be already formed by pre-weanling pups at PD18. However, at this early age, in the OPR task the allocentric memory expresses itself the preferential exploration of the objects at the familiar location rather than of the objects at the novel location as in adult rats. In fact, we found allocentric spatial memory that expressed itself in preferential exploration of the object at the novel location from adolescence onwards, i.e., in the PD38, PD48, and PD84 groups. Peri-adolescent (PD31) rats expressed a null preference in object exploration, but showed - as a more sensitive indicator of allocentric spatial memory - a clear preference to linger in the zone containing the object at the familiar location. Using both object related and non-object related measures of navigating behavior, we did not find any hints at the presence of allocentric representations in infantile rats at PD15.

Our findings demonstrate that pre-weanling rats at PD18, but not infant rats at PD15, are able to form rather stabile allocentric spatial representations that are maintained for up to 3 h after encoding. This finding seems to diverge from a foregoing study that revealed an earlier presence of spatial memory in the OPR task, i.e., around PD15 [16]. However, that study employed a distinctly shorter retention interval of only 5 min. Later onsets for the capability to form spatial memory were founded when memories were tested after longer retention intervals, e.g., at PD24 with a recall test after 2 h [14] and at PD26 h with a recall test after 24 h [15]. Contrasting with our results, in all these foregoing studies early OPR memory expressed itself in a preference for the object at the novel location i.e., as novelty preference. Our study is the first to show that initial OPR memory during development in rodents expresses itself in a preference for the object at the familiar location, i.e., as a familiarity preference. These effects which are in terms of Cohen's d of medium size (between 0.5 and 0.8) can be considered quite robust, considering that, not just the task per se, but also behavior during early development shows generally increased variability [31,32]. Moreover, our findings indicate that this familiarity preference does not depend on the length of the retention interval, but was likewise found for a 5-min and 3-h retention interval.

Notably, all of the previous studies showing in rats the presence of spatial representations based on preferential exploration of novelty during early development [14–16] included proximal cues inside the arena where the test was performed. Such proximal cues might have promoted an egocentric-spatial strategy instead of an allocentric-spatial strategy to solve the task [33]. Importantly, in our task procedure, no cues were disposed inside the arena and, additionally, the rats entered the open field during each trial of the encoding and recall phases (and even of the habituation sessions) from a different wall location. These features assured that the rats had to form an allocentric spatial representation to successfully navigate through the arena [19]. An advanced developmental onset of spatial navigation based on proximal cues and egocentric representations has been similarly described for the Morris Water Maze task [12,34]. Rats were already on PD17 able to use proximal cues to locate the safe platform. However, it was not until PD20 that the rats began to display signs of distal-cue utilization [11]. Altogether, these findings suggest that spatial navigation based on egocentric representations occurs earlier during development than allocentric based navigation [3,4].

Whereas the present findings are the first to indicate in rodents that during early development spatial memory expresses itself in a preference for familiarity, such preference for familiarity is well-known in human infants where it occurs with brief encoding times whereas with longer exposures the infant's attention turns to novelty [35]. In humans infants and children also a shift with age from familiarity preference to novelty preference has been observed, similar to the gradual shift from familiarity based to novelty based navigation in the allocentric version

of the OPR task which occurred in our rats between PD18 and PD25 [36]. Notably, the object zone discrimination index (OZDI) emerged as memory measure - indicating preferential lingering of the animal in the zone around the object at the familiar location - at nearly the age when the classical object discrimination index (ODI) exhibited a null preference (PD31), pointing out that this null preference reflects conflicting behavioral preferences rather than a lack of memory. Similar observations of a specific memory that is differently expressed at different ages with interleaving periods of latency have been found in human developmental studies [37,38].

It might be argued that familiarity preference in our younger rat pups reflects enhances levels of anxiety, as some studies showed a slower habituation in younger than older rats [39,40]. All rat groups in our study received the same and rather long time of habituation which, according to observations from pilot studies, presumably reduced anxiety induced by the arena to a minimum, even in the younger animals. In fact, a supplementary analysis a behavioral indicator of anxiety (i.e., the proportion of time during the recall phase the animals spent in the center of the arena vs close to the wall) revealed comparable values for the younger PD18, PD25 groups and the oldest PD84 group (Figure S3), which rules out anxiety as a main factor driving the familiarity preference in the younger animals. Rather than being insufficiently habituated to the arena, the separation from the mother per se might be the driving factor, i.e., that infant rats express preference towards familiarity might be basically linked to the fact that rodents, like humans, are altricial animals which perceive separation from the caregiver during early life as a danger signal. The transition from complete dependence on the caregiver to complete independence occurs during the first three weeks in rat pups and is accompanied by complex changes in responses to natural and learned threads, with corresponding changes in the supporting neural circuitry [41], that might ultimately also regulate the transition from familiarity to novelty based navigation in the OPR task during this period of development.

An important finding of our study is that infantile rats at PD15 did not show any behavioral signs of stable allocentric spatial representations. A possible explanation is that although the neural components to form spatial representation are present during infancy, they are not developed to a degree that allows the formation of integrated allocentric maps [6]. Alternatively, one could argue that, as the neural substrate for spatial representations (e.g., head direction cells, place cells, etc.) are present, infant rats are indeed also capable of forming corresponding spatial memory representations. But, the rats are not capable to use them for regulating their behavior [7,8]. Thus, the development of allocentric space representations might bear similarities with the learning of language where infants during the first year of life, based on sensory representations, are capable of discriminating a wide range of phonetic stimuli, but start to verbalize first words not before the end of the first year [42]. Also, our data do not exclude the possibility that PD15 pups form allocentric spatial representations but are able to maintain them for only short durations (i.e., < 3 h). Yet another explanation refers to the animal's motivation for spatial exploration. Although the exploratory motive toward familiar objects might already be present at PD15 [24], some more specific conditions might be required in order to trigger such exploration behavior.

5. Conclusion

Our data indicate that rats from PD18 onward are capable of forming stable allocentric memory representations that last for at least 3 h. Using an OPR task set up we show that initially these representations are expressed as preferential exploration of the object at the familiar location. This behavioral expression pattern gradually changes in the course of development over a null preference for any of the two objects in peri-adolescent rats (PD31) to preferential exploration of the object at the novel location from adolescence onward, i.e., in rats tested at PD38, PD48 and PD84. We did not find any behavioral sings for the presence of allocentric spatial representations before PD18, i.e., in pups tested at PD15, although the underlying neuronal circuitry is thought to be functioning at this age.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.bbr.2019.112013.

References

[1] C.V. Vorhees, M.T. Williams, Assessing spatial learning and memory in rodents, ILAR J. 55 (2014) 310–332, https://doi.org/10.1093/ilar/ilu013.
[2] A.W. Siegel, S.H. White, The development of spatial representations of large-scale environments, Adv. Child Dev. Behav. 10 (1975) 9–55, https://doi.org/10.1016/S0065-2407(08)60007-5.
[3] J.A. Ainge, R.F. Langston, Ontogeny of neural circuits underlying spatial memory in the rat, Front. Neural Circuits 6 (2012) 1–10, https://doi.org/10.3389/fncir.2012.00008.
[4] N. Burges, Spatial memory: how egocentric and allocentric combine, Trends Cogn Sci 10 (2006) 551–557, https://doi.org/10.1016/j.tics.2006.10.005.
[5] E.I. Moser, M.B. Moser, B.L. McNaughton, Spatial representation in the hippocampal formation: a history, Nat. Neurosci. 20 (2017) 1448–1464, https://doi.org/10.1038/nn.4653.
[6] H.M. Tan, T.J. Wills, F. Cacucci, The development of spatial and memory circuits in the rat, Wiley Interdiscip. Rev. Cogn. Sci. 8 (2017) 1–16, https://doi.org/10.1002/wcs.1424.
[7] H.M. Tan, J.P. Bassett, J. O'keefe, F. Cacucci, T.J. Wills, The development of the head direction system before eye opening in the rat, Curr. Biol. 25 (2015) 479–483, https://doi.org/10.1016/j.cub.2014.12.030.
[8] T.J. Wills, F. Cacucci, N. Burgess, J. O'keefe, Development of the hippocampal cognitive map in preweanling rats, Science 328 (2010) 1573–1576, https://doi.org/10.1126/science.1188224.
[9] K.G. Akers, A.D. Hamilton, Comparison of developmental trajectories for place and cue navigation in the Morris water task, Dev. Psychobiol. 49 (2007) 553–564, https://doi.org/10.1002/dev.20227.
[10] R.W. Brown, P.J. Kraemer, Ontogenetic differences in retention of spatial learning tested with Morris water maze, Dev. Psychobiol. 30 (1997) 329–341, https://doi.org/10.1002/(SICI)1098-2302(199705)30:4<329::AID-DEV6>3.0.CO;2-Q.
[11] J.W. Rudy, S.S. Morris, P. Albert, Ontogeny of spatial navigation in the rat: dissociation of "proximal"-and "distal-cue based behaviors, Behav. Neurosci. 101 (1987) 62–73, https://doi.org/10.1037/0735-7044.101.1.62.
[12] H.M. Carman, C.F. Mactutus, Proximal versus distal cue utilization in spatial navigation: the role of visual acuity? Neurobiol. Learn. Mem. 78 (2002) 332–346, https://doi.org/10.1006/nlme.2002.4062.
[13] A. Guskjolen, S.A. Josselyn, P.W. Frankland, Age-dependent changes in spatial memory retention and flexibility in mice, Neurobiol. Learn. Mem. 143 (2017) 59–66, https://doi.org/10.1016/j.nlm.2016.12.006.
[14] A. Travaglia, A.B. Steinmetz, J.M. Miranda, C.M. Alberini, Mechanisms of critical period in the hippocampus underlie object location learning and memory in infant rats, Learn. Mem. 25 (2018) 176–182, https://doi.org/10.1101/lm.046946.117.
[15] S.R. Westbrook, L.E. Brennan, M.E. Stanton, Ontogeny of object versus location recognition in the rat: acquisition and retention effects, Dev. Psychobiol. 56 (2014) 1492–1506, https://doi.org/10.1002/dev.21232.
[16] H.S. Krüger, M.D. Brackmann, J. Salamon, H. Ittrich, I.L. Hanganu-Opatz, Neonatal hippocampal lesion alters the functional maturation of the prefrontal cortex and the early cognitive development in pre-juvenile rats, Neurobiol. Learn. Mem. 97 (2012) 470–481, https://doi.org/10.1016/j.nlm.2012.04.001.
[17] R.F. Langston, E.R. Wood, Associative recognition and the hippocampus: differential effects of hippocampal lesions on object-place, object-context and object-place-context memory, Hippocampus 20 (2009) 1139–1153, https://doi.org/10.1002/hipo.20714.
[18] H. Eichenbaum, C. Stewart, R.G. Morris, Hippocampal representation in place learning, J. Neurosci. 10 (1990) 3531–3542, https://doi.org/10.1523/JNEUROSCI.10-11-03531.1990.
[19] D.E. Berlyne, Novelty and curiosity as determinants of explorative behavior, Br. J. Psychol. 41 (1950) 68–80, https://doi.org/10.1111/j.2044-8295.1950.tb00262.x.
[20] D.G. Mumby, S. Gaskin, M.J. Glenn, T.E. Schramek, H. Lehmann, Hippocampal damage and exploratory preferences in rats: memory for objects, places, and contexts, Learn. Mem. 9 (2002) 49–57, https://doi.org/10.1101/lm.41302.
[21] S. Gaskin, A. Gamliel, M. Tardif, E. Cole, D.G. Mumby, Incidental (unreinforced) and reinforced spatial learning in rats with ventral and dorsal lesions of the hippocampus, Behav. Brain Res. 202 (2009) 64–70, https://doi.org/10.1016/j.bbr.

2009.03.016.
[22] E.C. Warburton, M.W. Brown, Neural circuitry for rat recognition memory, Behav. Brain Res. 258 (2015) 131–139, https://doi.org/10.1016/j.bbr.2014.09.050.
[23] A. Ennaceur, One-trial object recognition in rats and mice: methodological and theoretical issues, Behav. Brain Res. 215 (2010) 244–254, https://doi.org/10.1016/j.bbr.2009.12.036.
[24] G.A. Goodwin, H. Yacko, Emergence of the exploratory motive in rats, Dev. Psychobiol. 45 (2004) 34–48, https://doi.org/10.1002/dev.20012.
[25] E. Mather, Novelty, attention, and challenges for developmental psychology, Front. Psychol. 1 (2013) 4–491, https://doi.org/10.3389/fpsyg.2013.00491.
[26] A.I. Ramsaran, H.R. Sanders, M.E. Stanton, Determinants of object-in-context and object-place-context recognition in the developing rat, Dev. Psychobiol. 58 (2016) 883–895, https://doi.org/10.1002/dev.21432.
[27] A.L. Ramsaran, S.R. Westbrook, M.E. Stanton, Ontogeny of object-in-context recognition in the rat, Behav. Brain Res. 298 (2016) 37–47, https://doi.org/10.1016/j.bbr.2015.04.011.
[28] I.Q. Whishaw, G. Mittleman, Visits to starts, routes, and places by rats (Rattus norvegicus) in swimming pool navigation tasks, J. Comp. Psychol. 100 (1986) 422–431, https://doi.org/10.1037/0735-7036.100.4.422.
[29] C. Chambon, N. Wegener, A. Gravius, W. Danysz, A new automated method to assess the rat recognition memory: validation of the method, Behav. Brain Res. 222 (2011) 151–157, https://doi.org/10.1016/j.bbr.2011.03.032.
[30] T. Ozawa, K. Yamada, Y. Ichitani, Long-term object location memory in rats: effects of sample phase and delay length in spontaneous place recognition test, Neurosci. Lett. 15 (2011) 37–41, https://doi.org/10.1016/j.neulet.2011.04.022.
[31] M.J. Anderson, G.W. Barnes, J.F. Briggs, K.M. Ashton, E.W. Moody, R.L. Joynes, D.C. Riccio, Effects of ontogeny on performance of rats in a novel object-recognition task, Psychol. Rep. 94 (2004) 437–443, https://doi.org/10.2466/pr0.94.2.437-443.
[32] K.E. Ameen-Ali, A. Easton, M.J. Eacott, Moving beyond standard procedures to assess spontaneous recognition memory, Neurosci. Biobehav. Rev. 53 (2015) 37–51, https://doi.org/10.1016/j.neubiorev.2015.03.013.
[33] M. Herbert, J. Bulla, D. Vivien, V. Agin, Are distal and proximal visual cues equally important during spatial learning in mice? A pilot study of overshadowing in the spatial domain, Front. Behav. Neurosci. 11 (2017) 1–8, https://doi.org/10.3389/fnbeh.2017.00109.
[34] H.M. Carman, R.M. Booze, C.F. Mactutus, Proximal versus distal cue utilization in preweanling spatial localization: the influence of cue number and location, Physiol. Behav. 79 (2003) 157–165, https://doi.org/10.1016/S0031-9384(03)00089-1.
[35] S.A. Rose, A.W. Gottfried, P. Melloy-Carminar, W.H. Bridger, Familiarity and novelty preferences in infant recognition memory: Implications for information processing, Dev. Psychol. 18 (1982) 704–713, https://doi.org/10.1037/0012-1649.18.5.704.
[36] C. Houston-Price, S. Nakai, Distinguishing novelty and familiarity effects in infant preference procedures, Infant Child Dev. 13 (2004) 341–348, https://doi.org/10.1002/icd.364.
[37] J.L. Richmond, J. Power, Age-related differences in memory expression during infancy: using eye-tracking to measure relational memory in 6- and 12-month-olds, Dev. Psychobiol. 56 (2014) 1341–1351, https://doi.org/10.1002/dev.21213.
[38] J. Koski, I.R. Olson, N.S. Newcombe, Tracking the eyes to see what children remember, Memory 21 (2013) 396–407, https://doi.org/10.1080/09658211.2012.735241.
[39] D.A. Feigley, P.J. Parsons, L.W. Hamilton, N.E. Spear, Development of habituation to novel environments in the rat, J. Comp. Physiol. Psychol. 79 (1972) 443–452, https://doi.org/10.1037/h0032842.
[40] S.E. File, The ontogeny of exploration in the rat: habituation and effects of handling, Dev. Psychobiol. 11 (1978) 321–328, https://doi.org/10.1002/dev.420110405.
[41] J. Debiec, R.M. Sullivan, The neurobiology of safety and threat learning in infancy, Neurobiol. Learn. Mem. 143 (2017) 49–58, https://doi.org/10.1016/j.nlm.2016.10.015.
[42] P. Kuhl, Early language acquisition: cracking the speech code, Nat. Rev. Neurosci. 5 (2004) 831–843, https://doi.org/10.1038/nrn1533.

Study 2 – Unfolding of spatial representation at systems level in infant rats.

Unfolding of spatial representation at systems level in infant rats

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Abstract

Spatial representations enable navigation from early life on. However, the brain regions essential to form spatial representations, like the hippocampus, are considered functionally immature before weaning. Here, we examined the formation of representations of space in rat pups on postnatal day (PD) 16, using a simple habituation paradigm where the pups were exposed to an arena on three occasions, separated by ~140 min. Whereas on the first two occasions the arena was the same, on the third 'test' occasion either proximal cues (Prox group), or distal cues (Dist group), or proximal and distal cues (Prox-Dist group), or no cues (No-change group) were rearranged. Locomotion (distance travelled) was used as behavioral measure of habituation, and c-Fos expression to measure regional brain activity at test. Locomotion generally decreased across the first two occasions. At test, it reached a minimum in the No-change group, indicating familiarity with the spatial conditions. By contrast, the Prox-Dist and Prox groups, but not the Dist group, displayed a significant increase in locomotion, indicating that the pups relied on proximal cues to explore spatial novelty. c-Fos activity in the No-change group was significantly suppressed in the hippocampus (CA1, CA3, dentate gyrus) but simultaneously enhanced in prelimbic (PL) medial prefrontal cortex, compared with untreated home-cage controls. Moreover, PL c-Fos activity negatively correlated with locomotion, suggesting that these habituated animals relied on a medial prefrontal cortex representation to suppress spatial locomotion. By contrast, in both Prox-Dist and Prox groups c-Fos activity was enhanced in hippocampal CA1 and CA3 regions. Moreover, the CA1 c-Fos activity positively correlated with locomotion, suggesting this region to be particularly involved in regulating exploration of spatial novelty. Our findings show that functional representations of space at a systems level are formed already in pre-weanling rats.

Key words

Spatial representation; Habituation; Proximal cues; Distal cues; Hippocampus; Medial prefrontal cortex; Pre-weanling rats; Development.

Introduction

Animals have the capability to navigate based on spatial maps that integrate information about environmental landmarks and their own movements. The spatial representations can be egocentric, defining spatial elements in relation to the own body, or allocentric, mapping spatial relationships among environmental elements independently of the own body position (Vorhees & Williams, 2014). In adult animals, a network essentially comprising hippocampal place cells, entorhinal grid cells and more distributed head direction cells is central for forming spatial representations, especially when allocentric (Moser et al., 2017).

However, when and how spatial representation emerges during early development is unclear. Recordings of neural activity provided consistent evidence that the different neural components of the spatial network are functioning already early during development even before extensive experience, i.e., in pre-weanling and infant rats before PD15 (Ainge & Langston, 2012; Tan et al., 2015, 2017; Wills et al., 2010). Recent findings suggest that, in addition to hippocampal and neighboring areas, other brain regions, specifically the medial prefrontal cortex (mPFC), also contribute to spatial navigation already early in life (Rinaldi et al., 2020). However, although the prefrontal-hippocampal spatial system may be functioning at the level of single neurons in pre-weanling rats, the involved regions are immature and it is unknown whether rats at this age can actually use this system for memory based navigation.

Behavioral studies in rats using the Morris water maze showed that allocentric spatial navigation emerges after PD20 while the egocentric strategy, tested in the visible platform version, emerges as early as PD17 (Akers & Hamilton, 2007; Rudy et al., 1987). In another test of hippocampus-dependent spatial abilities, the object place recognition task, successful performance emerged only from PD18 on (Contreras et al., 2019; Travaglia et al., 2018; Westbrook et al., 2014), except in one study showing functional spatial representation that were maintained over only a short 10-min interval already at PD16 (Krüger et al., 2012). Likewise, in the object-in-context recognition task which tests the animal´s capability to

process contextual cues and associates them with objects, rats at PD17 were able to acquire but not to retain these memories longer than 5 minutes (Sanders et al., 2020; Ramsaran et al., 2016). Thus, it is still an open question whether pre-weanling rats are able to form stable allocentric spatial representations that they use for navigation and last more than a few minutes.

In tasks, like the object place recognition task, rats do not properly explore the objects before PD18 (Contreras et al., 2019) possibly reflecting an inability to discriminate objects as separate entity, which makes such object-based tasks not suitable for testing spatial capabilities before this age. Therefore, to address the question whether pre-weanling rats navigate based on spatial representations, we used here a simple spatial habituation task. Habituation is a basic form of learning which describes the progressive decrease of the amplitude or frequency of a motor response to repeated sensory stimulation (Domjan, 2002). Our task relied on the pup's capability to explore the environment through locomotion and included two habituation sessions where the rat encountered the same spatial environment, and one test trial with either the same or novel spatial context configurations of proximal and distal cues (Bronstein et al., 1974; Feigley et al., 1972; O'Keefe & Nadel, 1978). Based on evidence that expression of the activity-regulated gene c-Fos sensitively differentiates brain areas involved in spatial tasks (Aggleton & Brown, 2005; Guskjolen et al., 2018; Tan et al., 2015; Wan et al., 1999), we used c-Fos activity to identify the network of brain regions the rat pup used for exploration during the test session. We provide evidence that reduced locomotion in a habituated spatial environment is regulated through the mPFC, whereas increased locomotion in a novel spatial environment involves hippocampal activation.

Materials and methods

Animals

A total of 66 male Long-Evans rats were used for the experiments. Animals were taken from 22 litters with each litter including 3 pups. All pups arrived (from Janvier, Le Genest-Saint-Isle, France) in our facilities on PD8 or PD9 which allow acclimatization for at least 3 days before any manipulation. The pups were maintained with their dam except during handling and behavioral tasks. All the behavioral tasks were performed on PD16 and all pups had opened their eyes and already started to explore their home cage surroundings on the day of the experiment. The pups were randomly assigned to 5 groups depending on the habituation task conditions (see below), i.e., the No-change (n = 21), Prox-Dist (n = 15), Prox (n = 12), Dist (n = 12); and Home-cage (n = 6) groups. The animal colony was kept at room temperature (22 ± 1°C) on a controlled 12 h light/12 h dark cycle (lights on at 7:00 h). All experimental procedures were performed in accordance with the European animal protection laws (Directive 2010/63/EU) and were approved by the Baden-Württemberg state authority.

Experimental procedures, design and task

All experimental procedures were performed between 7:00-16:00h, i.e., the light phase. For general habituation, each pup (of all groups) was carried in the home cage from the animal facility to the testing room and stayed there (in one of the room corners) together with the dam for 6 hours, once every day between PD12 and PD15. During this 6-hour room habituation period, the animals received 5 minutes of handling twice in order to diminish potential stress.

The spatial habituation task was conducted on PD16. The task consisted of two habituation sessions and one test session which were each separated by ~140 min (Figure 1A). The first two habituation sessions were identical and consisted each of a 10-min period where the pup could freely explore the open field with either the A or B context (counterbalance

within the group). The test session also lasted 10 min and differed depending on the experimental group, i.e., the No-change group was exposed to the same arena with the same proximal and distal cues as during the habituation sessions; for the Prox-Dist group novel proximal and distal cues were presented, for the Prox and Dist groups only the proximal and distal cues, respectively, were changed. The Home-cage control group did not undergo the spatial habituation task but, was kept in the home cage in the testing room during the corresponding intervals. At each session of the task, the animal was introduced in the arena from a different side, in order to facilitate an allocentric spatial representation (Langston & Wood, 2010). During the interval between sessions the pups were left undisturbed in the home cage with their dam and litter.

Apparatus and data reduction

The spatial habituation task was always performed in the same experimental room with the pup exposed to a circular open field arena. The arena was placed at the center of the room and surrounded by a circular black curtain, with the south side of the curtain used as entrance for the experimenter (X.S.). The arena had a diameter of 49 cm, a wall of 20 cm height and was made of grey PVC. The upper part of the arena was open, allowing the rat to perceive distal visual cues. For experimentally varying proximal cues a checkerboard pattern covered either the floor or the arena wall (Figure 1B). The distal cues consisted of four white tissues (100 cm x 80 cm and 50 cm x 80 cm) attached to the black curtain and 3 different tridimensional boxes supported by a wooden stick. Distal cues were not more than 200 cm away from the arena. To systematically vary proximal and distal cues between the conditions, the same cues were used but arranged in a different spatial configuration.

The animal's behavior was recorded by a video camera located above the center of the open field. Three fluorescent strip lights were placed on the floor of the room providing indirect light. White noise was presented at a constant intensity during all procedures to mask

any disturbing sounds. The open field was cleaned thoroughly between trials with 70% ethanol solution.

The rat's locomotor activity was scored offline using the ANY-Maze tracking software (Stoelting Europe, Dublin, Ireland). Distance travelled was tracked and calculated for each session. Spatial habituation was indicated by a decrease in locomotion (i.e., distance travelled) across two sessions that took place in the same context, and reflected that the animal had formed a spatial memory representation. Correspondingly, an increase in locomotion after introducing a change in proximal and/or distal context cues in the test phase indicated that the animal responded to context novelty based on a memory representation of the habituation sessions. We focused the analyses on the first minute of each session because it is most sensitive to novelty exploration (Winters et al., 2004).

c-Fos immunocytochemistry

After the test session the rats were returned to the home cage. Ninety minutes later, they were decapitated, and the brains were removed intact, rapidly frozen in metylbutane (Sigma Aldrich, Taufkirchen, Germany), and stored at -40°C. The 90-min interval corresponds to the time of peak production of c-Fos protein after an event initiation (Bisler et al., 2002; Zangenehpour & Chaudhuri, 2002). The subsequent procedures were as described previously (Mendez et al., 2015). The brains were cut in coronal serial sections (30 μm) at -20°C in a cryostat microtome (model HM 505-E, Microm International GmbH, Heidelberg, Germany). The sections were mounted on gelatinized slides, which were post-fixed in buffered 4% paraformaldehyde (0.1 M, pH 7.4) for 30 min and rinsed in phosphate-buffered saline (PBS) (0.01 M, pH 7.4). They were subsequently incubated for 15 min with 3% hydrogen peroxidase in PBS to remove endogenous peroxidase activity, and then washed twice in PBS. After blocking with PBS solution containing 10% Triton X-100 (PBS-T) (Sigma, USA) and 3% bovine serum albumin for 30 min, sections were incubated with a rabbit poly-clonal anti-

c-Fos solution (1: 10,000) (Santa Cruz Biotech, sc-52, USA) diluted in PBS-T for 24 h at 4°C in a humid chamber. Slides were then washed 3 times with PBS and incubated in a goat anti-rabbit biotinylated IgG secondary antibody (Pierce, USA; diluted 1:200 in incubating solution) for 2 h at room temperature. They were washed 3 times in PBS and reacted with avidin biotin per oxidase complex (Vectastain ABC Ultrasensitive Elite Kit, Pierce) for 1 h. After 2 washes in PBS, the reaction was visualized, treating the sections for 3 min in a commercial nickel-cobalt intensified diaminobenzidine kit (Pierce, USA). The reaction was terminated by washing the sections twice in PBS. Slides were then dehydrated through a series of graded alcohols, cleared with xylene, and cover-slipped with Entellan (Merck, USA) for microscopic evaluation. All immunocytochemistry procedures included sections that served as controls where the primary antibody was not added. Slides containing sections of a specific brain region were stained at the same time. Slides were coded so that the experimenters performing the entire analysis were blinded to the conditions of individual subjects.

Regions of interest (ROIs) were defined based on the literature about hippocampal and cortical regions known to be involved in the formation of spatial and episodic memories, and anatomically determined according to Paxinos and Watson's atlas (2005). ROIs and their distance (in mm) from bregma were: +2.2 mm for the prelimbic (PL), infralimbic (IL), and cingulate (CG) cortices; -2.0 mm for the hippocampal cornu ammonis 1 (CA1), cornu ammonis 3 (CA3) and dentate gyrus (DG) subfields; +1.4 mm for primary motor (M1) and primary somatosensory (S1) cortices; -2.0 mm for the agranular retrosplenial (RSA) and parietal (PAR) cortices; -4.0 mm for perirhinal (PRH) and entorhinal (ENT) cortices.

The number of c-Fos positive nuclei in ROIs was quantified in two alternate sections 30 μm apart. Quantification was performed by systematically sampling each of the regions selected using superimposed counting frames. Sizes of the counting frames ranged from 72,000 μm^2 (RSA) to 120,000 μm^2 (ENT and PRH). The total area sampled by these frames

per region in each section was: 140,000 μm^2 in PL, IL; 120,000 μm^2 in CG; 144,000 μm^2 in CA1, CA3; 80,000 μm^2 in DG; 140,000 μm^2 in M1 and S1 cortices; 72,000 μm^2 in RSA; 144,000 μm^2 in PAR; 120,000 μm^2 in PRH; 240,000 μm^2 in ENT. Cell counts were conducted using a microscope (Leica DFC490, Germany) coupled to a computer with software installed (Leica application suite, Germany). c-Fos positive nuclei were defined based on homogenous grey-black stained elements with a well-defined border. Finally, the mean count of two sections was calculated for each subject and region.

Statistical analyses

Statistical analyses were performed using SPSS software (IBM, Armonk, NY, USA). Analysis of travelling distance was based on a global analysis of variance (ANOVA) with a Group factor (No-change, Prox-Dist, Prox, Dist groups) and a repeated-measures factor Session (first, second habituation sessions, test sessions). The significant Group x Session interaction effect from this analysis was followed by one-way sub-ANOVA combined with pairwise LSD post hoc comparisons and paired-samples t-tests.

Values of c-Fos activity were likewise first analyzed by a global ANOVA including a Group factor (Home cage, No-change, Prox-Dist, Prox, Dist groups) and a repeated measures Area factor (PL, IL, CG, CA1, CA3, DG, M1, S1, RSA, PAR, PRH, ENT). The significant Group x Area interaction was followed by one-way ANOVA with a Group factor, performed separately for each area, combined with pairwise LSD post-hoc comparisons. For a functional connectivity analysis based on c-Fos activity, Pearson correlation coefficients were calculated between all pairs of brain areas as well as between each brain area and behavioral data, and the total number of significant correlations was compared between groups by chi-square test. Connectivity graphs were constructed using both c-Fos quantifications and correlation coefficients. The Igraph package (v1.2.4.2) in R (RStudio, Bonston, MA, USA) was used to visualize the networks.

In cases (of one way-ANOVA, t-tests) where normality of the distribution or equal group variances was not assured, we additionally used non-parametric test (Kruskal-Wallis H test, Wilcoxon-signed rank test, respectively), and only when these nonparametric tests confirmed significance, respective results are reported. Results are presented as means ± SEM. A $p < 0.05$ level (uncorrected for post-hoc pairwise comparisons) was considered significant.

Results

Behavior - travelling distance

As expected, distance travelled (in the 1st min of the exploration interval) uniformly decreased in all groups from the first to the second habituation sessions but, strongly differed between the groups at the test session depending on whether or not novel cue configurations were introduced ($F(6,112) = 2.557$, $p = 0.023$, for Group x Session interaction in the global ANOVA, Figure 1C). Analyses focusing on the first two habituation sessions confirmed the decrease in travelling distance occurring with the repeated exposure of the pups to the same environment, as a most robust phenomenon with no differences between the groups ($F(1, 56) = 92.087$, $p < 0.001$, for Session main effect; $p = 0.589$, and $p = 0.766$ for Group main effect and Group x Session interaction, respectively; $p < 0.003$ for separate pairwise comparison of Sessions in each Group). Yet, the groups showed quite differential travelling distances in the test session ($F(3,59) = 8.518$, $p < 0.001$, Figure 1D). With reference to the second habituation session, the No-change group further decreased locomotion ($p = 0.015$) indicating that the animals confronted to a third presentation of the same spatial configuration continued to habituate. By contrast, the Prox-Dist group showed a distinct increased in locomotion ($p = 0.029$), indicating that the pups discriminated the change in proximal and distal cue configurations. In the Prox group, travelled distance on average also increased from the second habituation session to the test session, although this was not significant ($p = 0.550$). Nevertheless, travelled distance at the test session was closely comparable in the Prox-Dist and Prox groups ($p = 0.375$), and both groups travelled distinctly longer distances than the No-change group ($p < 0.001$ and $p = 0.003$, respectively, Figure 1D). By contrast, travelling distance in the Dist group at the test session was very similar to that of the No-change group ($p = 0.706$). In combination, these data provide behavioral evidence that the pups form a representation of the spatial environment during the habituation sessions, that mediates a further down regulation of exploratory locomotion when, at the test session, the pup is

exposed to the same environment but that upregulates locomotion when novel proximal cue configurations are introduced. Distal cues are not involved in behavioral regulation.

Expression of c-Fos

We found major group differences in hippocampal and medial prefrontal cortical (mPFC) areas whereas primary motor and primary somatosensory cortices showed little changes ($F(15.715, 98.217) = 15.982$, $p < 0.001$ for global ANOVA Group x Area interaction, see Figure 2 for pairwise comparisons). Importantly, the No-change group expressed lower c-Fos activity in the hippocampal areas (CA1, CA3 and dentate gyrus, $p < 0.001$, $p = 0.015$, $p < 0.001$, respectively) but higher c-Fos activity in the prelimbic mPFC compared with the Home-cage group ($p = 0.003$). In contrast, in both Prox-Dist and Prox groups, c-Fos activity was enhanced in hippocampal CA1 and CA3 regions (all $p < 0.01$). Correlating c-Fos with locomotor activity at the test session revealed that c-Fos expression in the prelimbic cortex was negatively correlated with the distance travelled at test in the No-change group ($r = -0.910$, $p = 0.012$). However, a positive correlation was found between the c-Fos activity in CA1 and the distance travelled in the Prox-Dist ($r = 0.881$, $p = 0.020$) and Prox groups ($r = 0.947$, $p = 0.004$, Figure 2B), pointing towards an opposite functional role for hippocampal and mPFC areas in regulating spatial behavior at this age.

The Prox-Dist group, in mPFC regions displayed low levels of c-Fos activation, comparable to that in the Home-cage control group and distinctly lower than that of the No-change group ($p < 0.05$, for PL, IL, CG). Different from this pattern and similar to the No-change group, the Prox group showed increased c-Fos activity in mPFC regions (PL, IL, CG, $p < 0.05$ in comparison with the Home cage control group), overall suggesting that the mPFC response depends on an intermediate degree of novelty, i.e., a high response when only proximal cues are changed but no response when both proximal and distal cues change.

Interestingly, in the Dist group whose behavior during the test session did not differ from that of the No-change group, c-Fos activity was distinctly enhanced in the hippocampus (CA1, CA3 and dentate gyrus, all $p < 0.01$), retrosplenial ($p < 0.001$) and parietal ($p < 0.001$) regions compared to the Home-cage control and No-change groups. These increases indicate that the pups neuronally processed the spatial distal cues although not responding to them at the behavioral level.

We determined connectivity network graphs based on significant Pearson's correlation coefficients, to analyze the functional connectivity within the set of brain structures of interest and their correlation with locomotor behavior during the test session (Figure 3A). The graphs of the No-change and Prox-Dist groups were hallmarked by the negative correlation of PL cortex and the positive correlation of CA1, respectively, with travelled distance. Otherwise, the number of significant interregional correlations in these groups was low and did not differ from that of the Home-cage control group (Figure 3B). The number of interregional correlations was increased in the Prox group, when compared with the No-change group ($\chi2 = 9.167$, $p = 0.002$), with the majority of these connections involving the hippocampal CA1, CA3 and DG areas. Interestingly, the Dist group that did not behaviorally respond to the change in distal cue formation, was the group that displayed the strongest increase in the number of interregional correlations in c-Fos activity during the test session ($\chi2 > 9.570$, $p < 0.002$, for the comparisons with all other groups except the Prox group; Figure 3B). Unlike in the Prox group, in the Dist group these regional intercorrelations spared hippocampal areas but mainly connected mPFC and parietal cortices, on the one hand, with entorhinal and perirhinal cortices, and on the other hand, with the primary motor cortex. The correlations with entorhinal and perirhinal cortices were negative whereas the correlation with primary motor cortex were positive in direction.

Discussion

We tested whether pre-weanling infant rats at PD16 are already capable to form persisting (~140 min) spatial representations using a simple spatial habituation paradigm that allowed to assess behavioral (distance travelled during exploration) as well as neuronal (c-Fos activity at the test session) read-outs of spatial memory formation. Results indicate a robust decrease in distance travelled during exploration with the first repetition of exposing the rat pups to the identical arena environment (2nd Habituation session), followed by a further decrease in locomotion when the rat pup was exposed a third time to the identical environment (test session of the No-change group). By contrast, exposing the rat pup at the third session, i.e., the test session to an arena with changed proximal and distal cue configurations or only with changed proximal cues, invoked a strong increase in distance travelled during exploration. This increase was not observed when only the distal cue configuration was changed. In combination, this pattern indicates that the pups at PD16 indeed form a spatial representation that is used to differentially regulate exploratory behavior in familiar vs novel spatial environments, and that preferentially integrates proximal rather than distal spatial cues. This picture was corroborated by the analysis of regional c-Fos activity levels at the test session. Exposed a third time to the identical spatial environment, rat pups of the No-change group not only travelled the shortest distance during this session but also showed highest c-Fos activity in prelimbic mPFC areas with the c-Fos levels in this region being strongly negatively correlated with locomotor behavior. This finding supports the notion that inhibition of locomotor activity during spatial habituation is mediated through mPFC regions such as the prelimbic region, participating in the representation of space. We did not find signs of a hippocampal contribution to regulating habituation in the pups of the No-change group. By contrast, the rats which were exposed to changes in the configuration of distal and proximal or only proximal cues at the test session and which responded to these changes with an increase in locomotion, showed distinctly enhanced c-Fos activity levels in hippocampal areas, and the

increase in c-Fos in CA1 was moreover highly positively correlated with the distance travelled at the test session. These findings support the view that hippocampal regions particularly contribute to regulating explorative locomotion in response to novel proximal aspects of the spatial environment.

It can be excluded that the robust differences in locomotor and c-Fos activity between groups were strongly biased by maturational processes, as all experiments took place on the same day (PD16), and there were virtually no differences in locomotion between the groups at the two habituation sessions. Moreover, all groups including the home cage controls, showed very comparable c-Fos activity in primary motor cortex, and only minor differences in primary somatosensory cortex. Indeed, locomotion and associated exploratory skills, used here as a behavioral indicator of spatial memory, are in essence developed by the end of second postnatal week (Altman & Sudarshan, 1975). In comparison, the time when response habituation (to repeated stimulation) occurs during development appears to be more variable and depending on the type of stimulation (Bronstein et al., 1974; Einon et al., 1975; Feigley et al., 1972). The very robust decrease in locomotion we found here in all groups across the first two habituation sessions and, in the test session, specifically for the No-change group corroborates the view that habituation is in principle established before PD16. We also can exclude that locomotor responses were confounded by non-spatial aspects of the stimulus conditions, because for manipulating the proximal and distal context of the arena we only changed the spatial configuration of the cues but not the cues themselves. Moreover, to assure the formation of allocentric spatial representations, the pups entered the arena at each session from a different side.

Our findings demonstrating that pups can form persisting spatial representations already at PD16 extends previous studies where this capability emerged later during development. In tasks requiring the discrimination of objects or in the hidden platform version of the Morris water maze task, behavioral hints at the formation of enduring spatial

representations (persisting more than 10 min) were revealed in rat pups not before PD17 (Ainge & Langston, 2012; Akers & Hamilton, 2007; Contreras et al., 2019; Ramsaran et al., 2016; Rudy et al., 1987; Sanders et al., 2020; Westbrook et al., 2014). The earlier onset in the formation of spatial memory found here can be explained by the task that did not require discrimination of discrete objects and was performed in stress-free conditions. The earlier onset of behavioral signs of spatial memory, moreover, concurs with electrophysiological evidence indicating that the neuronal machinery of place, grid, and head direction cells in hippocampus and adjacent areas is well functioning at PD16 (Tan et al., 2015, 2017; Wills et al., 2010), although these structures as well as prefrontal structures contributing to spatial behavior are by far not fully matured at this age.

Our pups increased locomotion only when proximal cue configurations were changed but not when distal configurations changed, indicating that behavior is regulated via spatial representations only integrating proximal landmarks at this age. An earlier onset for the integration of proximal than distal cues into spatial navigation has been likewise found in previous studies using the Morris water maze task (Akers & Hamilton, 2007; Rudy et al., 1987) and the object-in-context learning task (Akers et al., 2011; Ramsaran et al., 2016). The pups neglecting distal cues might result from an immature visual acuity with pre-weanling rats not being able to discriminate cues in the distance (Carman et al., 2003; Carman & Mactutus, 2002). However, c-Fos levels in our Dist group provided ample evidence that these rats indeed processed the change in the configuration of distal cues (see below). Hence, rather than not perceiving these cues, it is more likely that the pups at PD16 are just unable to use them for regulating locomotor behavior.

The comparison of c-Fos activity levels between our Prox-Dist and No-change groups indicates that both hippocampus and mPFC contribute to the formation spatial representations, the former mediating increased locomotion to novel cue configurations, the later mediating habituation and suppression of locomotion once the actual environment has

been recognized as familiar. As to the hippocampus, our findings in pups concur with a great body of evidence in adult rodents identifying the hippocampus as key structure for navigation and the encoding of novel spatial representations (e.g., Klur et al., 2009; Loureiro et al., 2012; O'Keefe & Nadel, 1978). Similar to our findings in pups at PD16, adult rats showed increased hippocampal c-Fos activity during spatial learning on an object place recognition task as well as upon changes in the spatial configuration of familiar visual stimuli (Jenkins et al., 2004; Mendez et al., 2015; Wan et al., 1999). Moreover, like in our pups, c-Fos activity in CA1 in these adult rats was positively correlated with behavioral indicators of memory (Mendez et al., 2015). In rat pups muscimol-induced inactivation of the hippocampus prevented context fear learning at PD24 (Raineki et al., 2010). Hippocampal c-Fos activity was enhanced in pups at PD20 performing on the Morris water maze and object location task, whereas rats at PD16, the specific age we used here, did not show increased activation in both tasks (Comba et al., 2015). Likewise, hippocampal c-Fos activity was increased during contextual fear learning at PD24 but not at PD21 (Raineki et al., 2010). Against this backdrop, the enhanced hippocampal c-Fos activity in response to novel proximal cue configurations occurring in our rat pups much earlier during development, i.e., on PD16, can be well explained by our task paradigm allowing to more sensitively assess at this age the encoding of the specifically spatial aspects of the stimulation. Accordingly, around PD16 place cells in CA1 have been shown to generate new representations upon a novel context and reactivate familiar representations based on degraded stimuli (Muessig et al., 2016).

Our findings argue against the view derived from studies of adult rats, that hippocampus-based navigation selectively refers to distal cues (Piterkin et al., 2008; Ramsaran et al., 2016; Rudy et al., 1987). Indeed, the quite robust increases in c-Fos activity in hippocampal regions in our Prox group in combination with the strong correlation between CA1 c-Fos levels and locomotor activity in this group, support that proximal cues alone are sufficient to regulate hippocampus-based navigation at this age (Rinaldi et al., 2020).

Our data corroborates growing evidence that the formation of spatial representations involves the mPFC already early on in life, although these regions show a rather protracted developmental trajectory (Bitzenhofer et al., 2019; Chini et al., 2020). The profound increase in c-Fos levels in mPFC regions in the No-change group together with the negative correlation of c-Fos levels in the prelimbic regions of mPFC with locomotor activity supports the view that this region is central in mediating habituation towards familiar spatial environments, which is consistent with findings in adult rodents (e.g., Chudasama et al., 2012; Eichenbaum, 2017; Expósito et al., 2020). Interestingly, c-Fos levels in mPFC were not only enhanced in the No-change group but also in the Prox group showing increased locomotor behavior to the novel proximal cue configuration at the test session. This finding indicates that these areas beyond their involvement in spatial habituation, serve additional functions possibly in the regulation of attention (Birrell & Brown, 2000; Dejean et al., 2016; Hébert et al., 2017). The specific change of only the proximal cue configuration leaving distal cues unchanged might enforce attentional processes to resolve the seemingly conflictual context information (Hébert et al., 2017).

Notably, c-Fos levels in the Dist group indicated that the pups also processed the changes in the distal cue configuration, although this did not express in behavioral changes. Exposed to changes only in the distal cue configuration, the pups showed maximal c-Fos levels in parietal and retrosplenial areas, i.e., interconnected regions well-known to be centrally involved in regulating navigation based on distal cues and allocentric reference frames in the mature brain (Auger et al., 2012; Clark et al., 2018; Mitchell et al., 2018; Vann & Aggleton, 2005). Moreover, the Dist group showed prominently increased overall functional connectivity between brain regions. Surprisingly, this increase in functional connectivity appeared to spare hippocampal regions which may explain that the processing of distal cue changes was not integrated into the Dist group’s locomotor behavior.

In conclusion, our study shows that infant rats at PD16 which is typically the first day they show reliable exploratory locomotor behavior, are able to form persisting spatial representations. Discriminating between decreases in locomotion (habituation) to familiar spatial contexts and increases in locomotion to novel contexts, we provide evidence that these spatial representations are formed at a systems level including not only hippocampal regions regulating behavior to novelty, but also mPFC regions - particularly the prelimbic region - which appear to be centrally involved in locomotor inhibition and habituation to familiar environments and, additionally, in attentional control to conflictual spatial information. This systems view concurs with finding in adult rats, e.g., of impaired spatial learning (in the Morris water maze) after lesions to both the prelimbic mPFC as well as to the hippocampus (Wang & Cai, 2008). Coordinate phase-locked oscillatory and spike activity between hippocampal and prefrontal cortical regions emerges already within the first two weeks after birth (Brockmann et al., 2011). The present c-Fos data provide additional support for this systems view, in showing that the central structures interfacing the prelimbic mPFC with hippocampal CA1 and CA3 regions, i.e., perirhinal and (lateral) entorhinal cortices (Eichenbaum, 2017), displayed significantly enhanced c-Fos activity in both the pups exposed to a highly familiar environment (No-change group) as well as in the pups exposed to clear changes in the spatial context (Prox-Dist group, Figure 2). Collectively, our findings allocating complementary roles to mPFC and hippocampus in mediating responses to familiar and novel spatial contexts, respectively, provides evidence that the mPFC-hippocampal circuit might operate at a systems level during spatial encoding already at PD16. An obvious question arising here is, to what extent such function of the mPFC-hippocampal circuit generalizes already during this early stage of development to other domains of episodic memory formation (Takehara-Nishiuchi, 2020).

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Conflict of interests

The authors declare no conflict of interests.

Data Availability Statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

Aggleton, J. P., & Brown, M. W. (2005). Contrasting Hippocampal and Perirhinalcortex Function using Immediate Early Gene Imaging. *The Quarterly Journal of Experimental Psychology Section B*, *58*(3-4b), 218–233. https://doi.org/10.1080/02724990444000131

Ainge, J. A., & Langston, R. F. (2012). Ontogeny of neural circuits underlying spatial memory in the rat. *Frontiers in Neural Circuits*, *6*, 8. https://doi.org/10.3389/fncir.2012.00008

Akers, K. G., Candelaria-Cook, F. T., Rice, J. P., Johnson, T. E., & Hamilton, D. A. (2011). Cued platform training reveals early development of directional responding among preweanling rats in the Morris water task. *Developmental Psychobiology*, *53*(1), 1–12. https://doi.org/10.1002/dev.20480

Akers, K. G., & Hamilton, D. A. (2007). Comparison of developmental trajectories for place and cued navigation in the Morris water task. *Developmental Psychobiology*, *49*(6), 553–564. https://doi.org/10.1002/dev.20227

Altman, J., & Sudarshan, K. (1975). Postnatal development of locomotion in the laboratory rat. *Animal Behaviour*, *23*(4), 896–920. https://doi.org/10.1016/0003-3472(75)90114-1

Auger, S. D., Mullally, S. L., & Maguire, E. A. (2012). Retrosplenial Cortex Codes for Permanent Landmarks. *PLoS ONE*, *7*(8), e43620. https://doi.org/10.1371/journal.pone.0043620

Birrell, J. M., & Brown, V. J. (2000). Medial frontal cortex mediates perceptual attentional set shifting in the rat. *Journal of Neuroscience*, *20*(11), 4320–4324. https://doi.org/10.1523/jneurosci.20-11-04320.2000

Bisler, S., Schleicher, A., Gass, P., Stehle, J. H., Zilles, K., & Staiger, J. F. (2002). Expression of c-Fos, ICER, Krox-24 and JunB in the whisker-to-barrel pathway of rats: Time course of induction upon whisker stimulation by tactile exploration of an enriched environment. *Journal of Chemical Neuroanatomy*, *23*(3), 187–198. https://doi.org/10.1016/S0891-

0618(01)00155-7

Bitzenhofer, S., Pöpplau, J., Chini, M., Marquardt, A., & Hanganu-Opatz, I. (2019). Transient developmental increase of prefrontal activity alters network maturation and causes cognitive dysfunction in adult mice. *BioRxiv*, 558957. https://doi.org/10.1101/558957

Brockmann, M. D., Pöschel, B., Cichon, N., & Hanganu-Opatz, I. L. (2011). Coupled Oscillations Mediate Directed Interactions between Prefrontal Cortex and Hippocampus of the Neonatal Rat. *Neuron*, *71*(2), 332–347. https://doi.org/10.1016/j.neuron.2011.05.041

Bronstein, P. M., Neiman, H., Wolkoff, F. D., & Levine, M. J. (1974). The development of habituation in the rat. *Animal Learning & Behavior*, *2*(2), 92–96. https://doi.org/10.3758/BF03199129

Carman, H. M., Booze, R. M., Snow, D. M., & Mactutus, C. F. (2003). Proximal versus distal cue utilization in preweanling spatial localization: The influence of cue number and location. *Physiology and Behavior*, *79*(2), 157–165. https://doi.org/10.1016/S0031-9384(03)00089-1

Carman, H. M., & Mactutus, C. F. (2002). Proximal versus distal cue utilization in spatial navigation: The role of visual acuity? *Neurobiology of Learning and Memory*, *78*(2), 332–346. https://doi.org/10.1006/nlme.2002.4062

Chini, M., Pöpplau, J. A., Lindemann, C., Carol-Perdiguer, L., Hnida, M., Oberländer, V., Xu, X., Ahlbeck, J., Bitzenhofer, S. H., Mulert, C., & Hanganu-Opatz, I. L. (2020). Resolving and Rescuing Developmental Miswiring in a Mouse Model of Cognitive Impairment. *Neuron*, *105*(1), 60-74.e7. https://doi.org/10.1016/j.neuron.2019.09.042

Chudasama, Y., Doobay, V. M., & Liu, Y. (2012). Hippocampal-prefrontal cortical circuit mediates inhibitory response control in the rat. *Journal of Neuroscience*, *32*(32), 10915–10924. https://doi.org/10.1523/JNEUROSCI.1463-12.2012

Clark, B. J., Simmons, C. M., Berkowitz, L. E., & Wilber, A. A. (2018). The retrosplenial-

parietal network and reference frame coordination for spatial navigation. *Behavioral Neuroscience*, *132*(5), 416–429. https://doi.org/10.1037/bne0000260

Comba, R., Gervais, N., Mumby, D., & Holahan, M. (2015). Emergence of spatial behavioral function and associated mossy fiber connectivity and c-Fos labeling patterns in the hippocampus of rats. *F1000Research*, *4*, 396. https://doi.org/10.12688/f1000research.6822.1

Contreras, M. P., Born, J., & Inostroza, M. (2019). The expression of allocentric object-place recognition memory during development. *Behavioural Brain Research*, *372*,112013. https://doi.org/10.1016/j.bbr.2019.112013

Dejean, C., Courtin, J., Karalis, N., Chaudun, F., Wurtz, H., Bienvenu, T. C. M., & Herry, C. (2016). Prefrontal neuronal assemblies temporally control fear behaviour. *Nature*, *535*(7612), 420–424. https://doi.org/10.1038/nature18630

Domjan, M. (2002). *The principles of learning and behavior*. Stamford: Cenage Learning.

Eichenbaum, H. (2017). Prefrontal-hippocampal interactions in episodic memory. *Nature Reviews Neuroscience*, *18*(9), 547–558. https://doi.org/10.1038/nrn.2017.74

Einon, D., Morgan, M. J., & Sahakian, B. J. (1975). The development of intersession habituation and emergence in socially reared and isolated rats. *Developmental Psychobiology*, *8*(6), 553–559. https://doi.org/10.1002/dev.420080613

Expósito, A. N., Morillas, E., Gómez-Chacón, B., & Gallo, M. (2020). Prefrontal cortex activity patterns during taste neophobia habituation in adult and aged rats. *Behavioural Brain Research*, *392*, 112717. https://doi.org/10.1016/j.bbr.2020.112717

Feigley, D. A., Parsons, P. J., Hamilton, L. W., & Spear, N. E. (1972). Development of habituation to novel environments in the rat. *Journal of Comparative and Physiological Psychology*, *79*(3), 443–452. https://doi.org/10.1037/h0032842

Guskjolen, A., Kenney, J. W., de la Parra, J., Yeung, B. ru A., Josselyn, S. A., & Frankland, P. W. (2018). Recovery of “Lost” Infant Memories in Mice. *Current Biology*, *28*(14), 2283

– 2290.e3. https://doi.org/10.1016/j.cub.2018.05.059

Hébert, M., Bulla, J., Vivien, D., & Agin, V. (2017). Are distal and proximal visual cues equally important during spatial learning in mice? A pilot study of overshadowing in the spatial domain. *Frontiers in Behavioral Neuroscience*, *11*, 109. https://doi.org/10.3389/fnbeh.2017.00109

Jenkins, T. A., Amin, E., Pearce, J. M., Brown, M. W., & Aggleton, J. P. (2004). Novel spatial arrangements of familiar visual stimuli promote activity in the rat hippocampal formation but not the parahippocampal cortices: A c-fos expression study. *Neuroscience*, *124*(1), 43–52. https://doi.org/10.1016/j.neuroscience.2003.11.024

Klur, S., Muller, C., De Vasconcelos, A. P., Ballard, T., Lopez, J., Galani, R., Certa, U., & Cassel, J. C. (2009). Hippocampal-dependent spatial memory functions might be lateralized in rats: An approach combining gene expression profiling and reversible inactivation. *Hippocampus*, *19*(9), 800–816. https://doi.org/10.1002/hipo.20562

Krüger, H. S., Brockmann, M. D., Salamon, J., Ittrich, H., & Hanganu-Opatz, I. L. (2012). Neonatal hippocampal lesion alters the functional maturation of the prefrontal cortex and the early cognitive development in pre-juvenile rats. *Neurobiology of Learning and Memory*, 97(4), 470–481. https://doi.org/10.1016/j.nlm.2012.04.001

Langston, R. F., & Wood, E. R. (2010). Associative recognition and the hippocampus: Differential effects of hippocampal lesions on object-place, object-context and object-place-context memory. *Hippocampus*, *20*(10), 1139-1153. https://doi.org/10.1002/hipo.20714

Loureiro, M., Lecourtier, L., Engeln, M., Lopez, J., Cosquer, B., Geiger, K., Kelche, C., Cassel, J. C., & Pereira De Vasconcelos, A. (2012). The ventral hippocampus is necessary for expressing a spatial memory. *Brain Structure and Function*, *217*(1), 93–106. https://doi.org/10.1007/s00429-011-0332-y

Mendez, M., Arias, N., Uceda, S., & Arias, J. L. (2015). C-Fos expression correlates with

performance on novel object and novel place recognition tests. *Brain Research Bulletin*, *117*, 16–23. https://doi.org/10.1016/j.brainresbull.2015.07.004

Mitchell, A. S., Czajkowski, R., Zhang, N., Jeffery, K., & Nelson, A. J. D. (2018). Retrosplenial cortex and its role in spatial cognition. *Brain and Neuroscience Advances*, *2*, 239821281875709. https://doi.org/10.1177/2398212818757098

Moser, E. I., Moser, M. B., & McNaughton, B. L. (2017). Spatial representation in the hippocampal formation: A history. *Nature Neuroscience*, *20*(11), 1448–1464. https://doi.org/10.1038/nn.4653

Muessig, L., Hauser, J., Wills, T. J., & Cacucci, F. (2016). Place Cell Networks in Pre-weanling Rats Show Associative Memory Properties from the Onset of Exploratory Behavior. *Cerebral Cortex*, *26*(8), 3627–3636. https://doi.org/10.1093/cercor/bhw174

O'Keefe, J., & Nadel, L. (1978). *The hippocampus as a cognitive map*.. Oxford University Press.

Piterkin, P., Cole, E., Cossette, M. P., Gaskin, S., & Mumby, D. G. (2008). A limited role for the hippocampus in the modulation of novel-object preference by contextual cues. *Learning and Memory*, *15*(10), 785–791. https://doi.org/10.1101/lm.1035508

Raineki, C., Holman, P. J., Debiec, J., Bugg, M., Beasley, A., & Sullivan, R. M. (2010). Functional emergence of the hippocampus in context fear learning in infant rats. *Hippocampus*, *20*(9), 1037-1046. https://doi.org/10.1002/hipo.20702

Ramsaran, A. I., Westbrook, S. R., & Stanton, M. E. (2016). Ontogeny of object-in-context recognition in the rat. *Behavioural Brain Research*, *298*, 37–47. https://doi.org/10.1016/j.bbr.2015.04.011

Rinaldi, A., De Leonibus, E., Cifra, A., Torromino, G., Minicocci, E., De Sanctis, E., López-Pedrajas, R. M., Oliverio, A., & Mele, A. (2020). Flexible use of allocentric and egocentric spatial memories activates differential neural networks in mice. *Scientific*

Reports, *10*(1), 1–15. https://doi.org/10.1038/s41598-020-68025-y

Rudy, J. W., Stadler-Morris, S., & Albert, P. (1987). Ontogeny of Spatial Navigation Behaviors in the Rat: Dissociation of "Proximal"-and "Distal"-Cue-Based Behaviors. *Behavioral Neuroscience*, *101*(1), 62–73. https://doi.org/10.1037/0735-7044.101.1.62

Sanders, H. R., Heroux, N. A., & Stanton, M. E. (2020). Infant rats can acquire, but not retain contextual associations in object-in-context and contextual fear conditioning paradigms. *Developmental Psychobiology*, *January*, 1–7. https://doi.org/10.1002/dev.21980

Takehara-Nishiuchi, K. (2020). Prefrontal–hippocampal interaction during the encoding of new memories. *Brain and Neuroscience Advances*, *4*, 239821282092558. https://doi.org/10.1177/2398212820925580

Tan, H. M., Bassett, J. P., O'Keefe, J., Cacucci, F., & Wills, T. J. (2015). The development of the head direction system before eye opening in the rat. *Current Biology*, *25*(4), 479–483. https://doi.org/10.1016/j.cub.2014.12.030

Tan, H. M., Wills, T. J., & Cacucci, F. (2017). The development of spatial and memory circuits in the rat. *Wiley Interdisciplinary Reviews: Cognitive Science*, *8*(3), 1–16. https://doi.org/10.1002/wcs.1424

Travaglia, A., Steinmetz, A. B., Miranda, J. M., & Alberini, C. M. (2018). Mechanisms of critical period in the hippocampus underlie object location learning and memory in infant rats. *Learning and Memory*, *25*(4), 176–182. https://doi.org/10.1101/lm.046946.117

Vann, S. D., & Aggleton, J. P. (2005). Selective dysgranular retrosplenial cortex lesions in rats disrupt allocentric performance of the radial-arm maze task. *Behavioral Neuroscience*, *119*(6), 1682–1686. https://doi.org/10.1037/0735-7044.119.6.1682

Vorhees, C. V., & Williams, M. T. (2014). Assessing spatial learning and memory in rodents. *ILAR Journal*, *55*(2), 310–332. https://doi.org/10.1093/ilar/ilu013

Wan, H., Aggleton, J. P., & Brown, M. W. (1999). Different Contributions of the Hippocampus and Perirhinal Cortex to Recognition Memory. *The Journal of*

Neuroscience, *19*(3), 1142–1148. https://doi.org/10.1523/JNEUROSCI.19-03-01142.1999

Wang, G. W., & Cai, J. X. (2008). Reversible disconnection of the hippocampal-prelimbic cortical circuit impairs spatial learning but not passive avoidance learning in rats. *Neurobiology of Learning and Memory*, *90*(2), 365–373. https://doi.org/10.1016/j.nlm.2008.05.009

Westbrook, S. R., Brennan, L. E., & Stanton, M. E. (2014). Ontogeny of object versus location recognition in the rat: Acquisition and retention effects. *Developmental Psychobiology*, *56*(7), 1492–1506. https://doi.org/10.1002/dev.21232

Wills, T. J., Cacucci, F., Burgess, N., & O'Keefe, J. (2010). Development of the Hippocampal Cognitive Map in Preweanling Rats. *Science*, *328*(5985), 1573–1576. https://doi.org/10.1126/science.1188224

Winters, B. D., Forwood, S. E., Cowell, R. A., Saksida, L. M., & Bussey, T. J. (2004). Double dissociation between the effects of peri-postrhinal cortex and hippocampal lesions on tests of object recognition and spatial memory: Heterogeneity of function within the temporal lobe. *Journal of Neuroscience*, *24*(26), 5901–5908. https://doi.org/10.1523/JNEUROSCI.1346-04.2004

Zangenehpour, S., & Chaudhuri, A. (2002). Differential induction and decay curves of c-fos and zif268 revealed through dual activity maps. *Molecular Brain Research*, *109*(1–2), 221–225. https://doi.org/10.1016/S0169-328X(02)00556-9

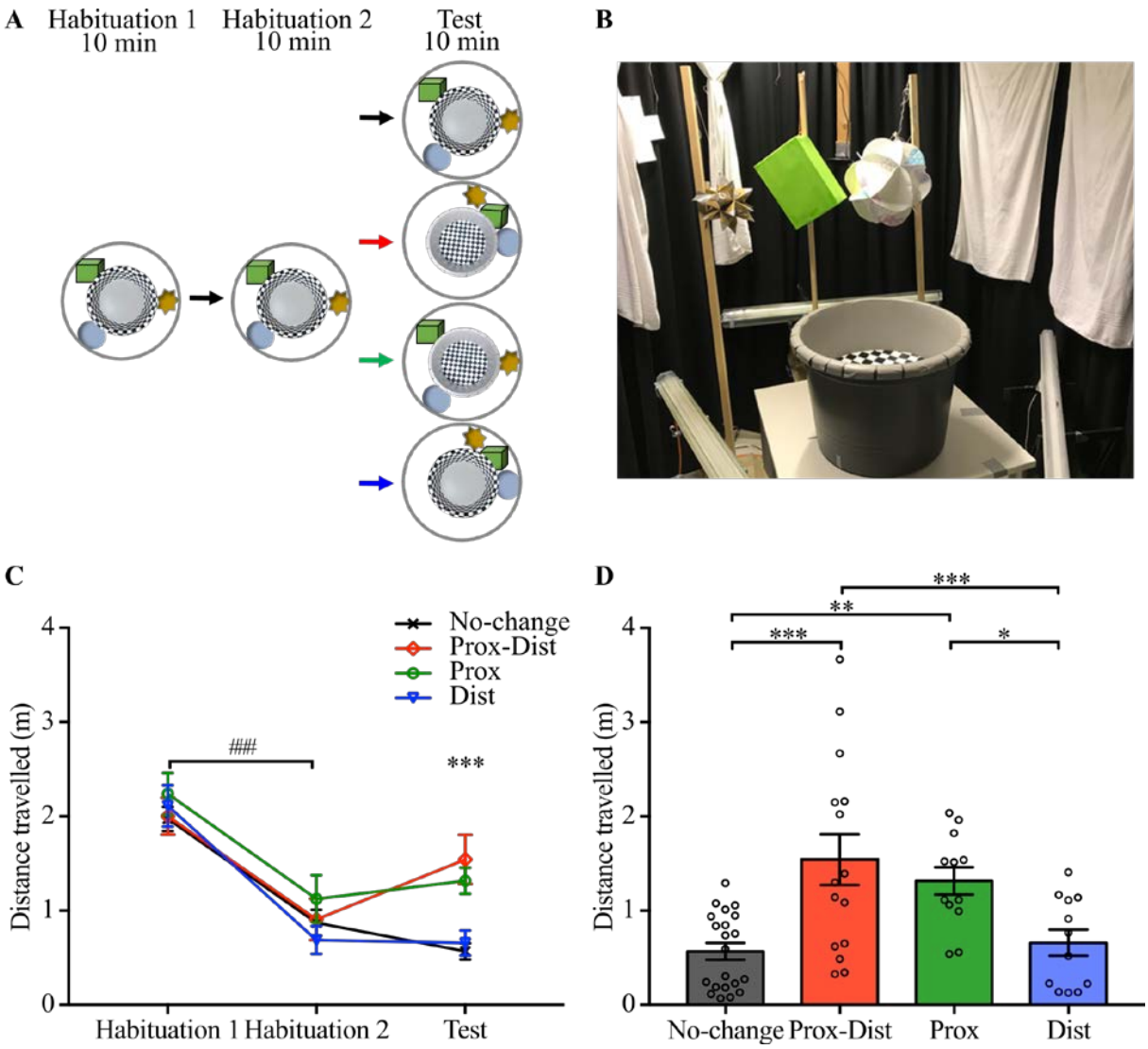

FIGURE 1. Experimental design and locomotor behavior. (A) The behavioral experiments included 4 groups which were all tested on 2 habituation sessions and 1 test session, with each session allowing the rat to explore the open field arena for 10 min. Sessions were separated by a ~140 min interval during which the pups were returned to the home cage with their dam. The arenas in the two habituation sessions were identical. On the test session, the No-change group (n = 21) was again exposed to the same arena, for the Prox-Dist group (n =15) proximal and distal cues were reconfigured, for the Prox group (n = 12) only proximal cues, and for the Dist group (n = 12) only distal cues. An additional Home-cage control group (n = 6) where the pups remained in their home cage during sessions was used only as control for c-Fos

activity and is not shown here. (B) Photo of the arena illustrating the experimental proximal cues (checkerboard pattern covering either the floor or the wall of the arena) and the distal cues (4 white tissues and 3 boxes presented in different spatial configurations). (C) Mean (±SEM) distance travelled for each group during 1st minute of habituation and test sessions (###, *** $p < 0.001$, ANOVA main effect across Habituation session and across Groups for the Test session, respectively) and (D) separately for the test session (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, for pairwise comparison).

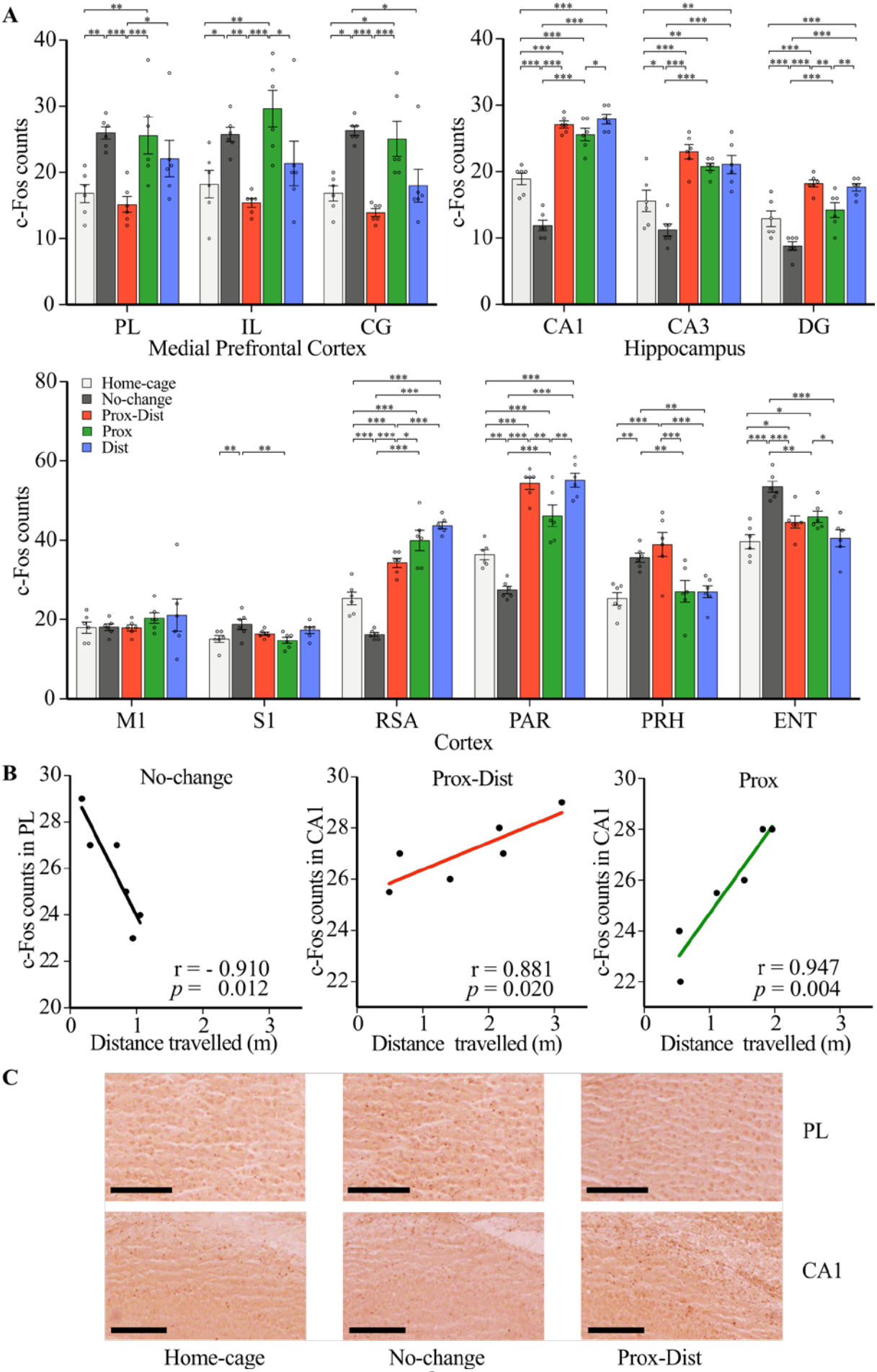
A
c-Fos counts
PL
IL
CG
Medial Prefrontal Cortex
CA1
CA3
DG
Hippocampus
Home-cage
No-change
Prox-Dist
Prox
Dist
M1
S1
RSA
PAR
PRH
ENT
Cortex
B
No-change
c-Fos counts in PL
r = - 0.910
p = 0.012
Distance travelled (m)
Prox-Dist
c-Fos counts in CA1
r = 0.881
p = 0.020
Prox
r = 0.947
p = 0.004
C
PL
CA1
Home-cage
No-change
Prox-Dist

FIGURE 2. (A) Mean (±SEM) counts of c-Fos positive cells in studied brain regions, top left: medial prefrontal cortex including prelimbic (PL), infralimbic (IL), and cingulate cortices (CG), and (top right) hippocampal subfields CA1, CA3 and dentate gyrus (DG). Bottom: primary motor and primary somatosensory cortices (M1 and S1), agranular retrosplenial (RSA), parietal (PAR), perirhinal (PRH), entorhinal (ENT) cortices. *$p < 0.05$, ** $p < 0.01$ and ***$p < 0.001$ for pairwise comparisons between experimental groups. (B) Correlations of locomotion (distance travelled) with c-Fos counts (left) in the PL region of mPFC in the No-change group, and (middle and right) in CA1 in the Prox-Dist and Prox groups. (C) Representative images of c-Fos staining selected for cell count analysis in PL and CA1 (scale bar: 200 µm).

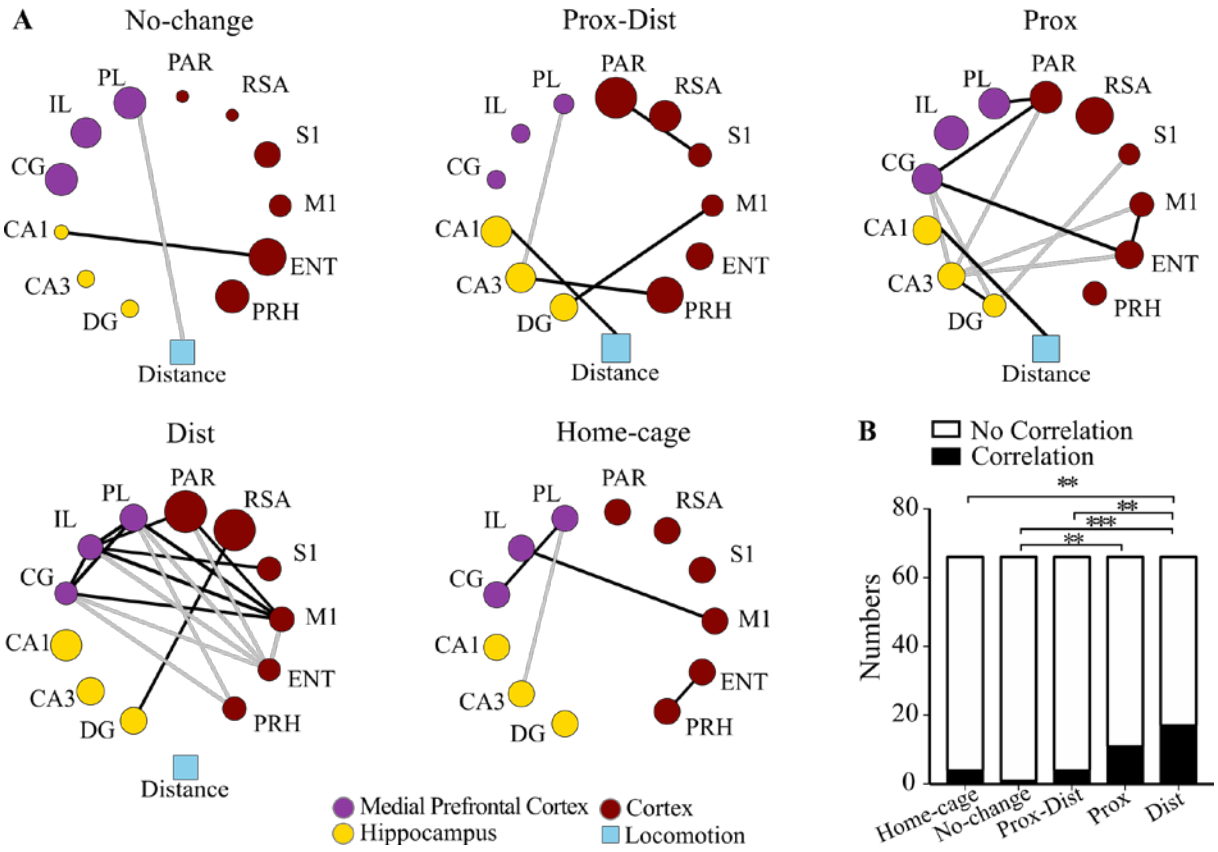

FIGURE 3. (A) Network connectivity analysis of c-Fos activity in (top) No-change, Prox-Dist, Prox groups, and (bottom) Dist and Home-cage control groups. Graphs include correlations with distance travelled (Distance, blue square) during the test session, and indicate only significant ($p < 0.05$) r values (positive - black, negative - grey connection lines). Brain regions are color-grouped and node size is proportional to c-Fos activity, with activity of the Home-cage group taken as baseline. (B) Number of significant correlations (black bars) shown as proportion of the total number of possible correlations (i.e., n = 66) between brain regions for the 5 groups (**$p < 0.01$, *** $p < 0.001$, for pairwise comparison with $\chi 2$ test).

Study 3 – Discrete spatial experiences during infancy builds schema memory for adult learning.

Discrete spatial experience during infancy builds schema memory for adult learning

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Abstract

Adult behavior is commonly thought to be shaped by early-life experience, but paradoxically, episodes experienced during infancy are forgotten. Exposing rats during infancy to discrete spatial configurational changes, we show that the rats in adulthood were significantly better at forming persistent spatial memory than were control rats with only non-spatial infantile experience, while the infantile episode itself was not retrievable. Infantile spatial experience increased c-Fos activity at memory testing during adulthood in prelimbic medial prefrontal cortex, whereas hippocampal activity remained unchanged. Adult spatial memory capability only benefitted from spatial experience occurring during the sensitive period of infancy, but not when occurring later during childhood, and when sleep followed the infantile experience. In conclusion, rather than forming lasting hippocampal episodic memory, infantile spatial experience, by a sleep-dependent mechanism, favors the formation of persisting cortical schema memories that facilitate learning in adulthood.

Keywords

Systems memory consolidation, Spatial memory development, Medial prefrontal cortex, Infantile amnesia, Sensitive period, Sleep

Introduction

Early life experience critically forms behavior in adulthood. This is a long-standing and prominent cultural idea that has been at the core of modern psychology as well as recent research linking, for example, traumatic experience during early life with capabilities to cope with stress in adulthood (Lupien et al., 2009). However, the importance of early experience for adult behavior stands in stark contrast with the observation that the memories formed of the multiple episodes during infancy are altogether easily and rapidly forgotten, a phenomenon known as infantile amnesia (Akers et al., 2014; Josselyn and Frankland, 2012). A proposal reconciling these apparently diverging observations is, that rather than being forgotten, episodic memories formed of experience during infancy are transformed into more general schema-like memories. These schema memories, then serve as enduring supraordinate knowledge to facilitate behavioral adaptation during adulthood (Ramsaran et al., 2019; Sekeres et al., 2018; Moscovitch et al., 2016; Frankland and Bontempi, 2005). The transformation into schema representation may make the original episodic memory less accessible, or even promote its forgetting.

Despite the outstanding theoretical interest, amazingly little experimental work has been performed on how a certain separate episodic experience during infancy is transformed to feed into adult knowledge systems (Donato et al., 2020; Ramsaran et al., 2019; Keresztes et al., 2018; Alberini and Travaglia, 2017). Here we took advantage of the well-controlled conditions of a rat model, to show that non-emotional episodes, i.e., the exposure to discrete changes in a spatial configuration for only a short interval on 4 days during a rat's infancy – when the hippocampus is still immature - strongly impacts the rat's spatial learning ability during adulthood. To induce spatial experience during infancy, we employed a simple procedure: the rats of a **Spatial-experience** group were placed in an arena with two identical objects for 5 min. Then, after a 5-

min break, the rats re-entered the arena for another 5-min; however, this time one of the objects was moved to another location (Figure 1A). A control group of rats was exposed to **Non-spatial experience** during infancy by only changing the kind of one of the two objects between the two 5-min exposure periods, instead of its spatial configuration. An additional **No-experience** control group did not undergo experimental arena visits during infancy. During adulthood, around postnatal day (PD) 80, all rats were tested on a classical object place-recognition (OPR) task, with a 3-hour delay between encoding and retrieval testing, to assess the animal's capability to form persisting spatial representations.

Material and methods

Animals

A total of 144 male Long-Evans rats were used for the experiments. Rat pups were allocated to the different experimental group such that each group derived from 2-3 litters of 4-6 pups. In total 25 litters were used for the whole experiment. Two litters were born in our own animal facilities (each litter was culled to 4 pups one or two days after parturition). The remaining pups arrived (from Janvier, Le Genest-Saint-Isle, France) in our facilities at least 4 days before any manipulation in order to allow acclimatization. It was ensured by inspection that all pups had opened their eyes and already started to explore their home cage surroundings on the day the experimental procedures started. The pups were maintained with their dam until weaning (PD21). The animal colony was kept at a room temperature of 22±1 °C, on a 12 h/12 h light/dark cycle (lights on at 6:00 h). All rats had free access to food and water throughout the experiments. All experimental procedures were performed in accordance with the European animal protection laws (Directive 2010/63/EU, European Community) and were approved by the Baden-Württemberg state authority.

Experimental groups

The 3 groups of the main experiments were the Spatial-experience group (n = 18 pups), the Non-spatial experience group (n = 18 pups), and the No-experience group (n = 18 pups). To test for long-term episodic memory for early spatial experience, a separate group (Long-term OPR, n = 12 pups) was exposed to a single encoding phase on the OPR at PD24 and tested at adulthood. Another control group included rats tested at adulthood in a different context (Context-change group, n = 12 pups). Exposure to spatial and non-spatial experience took place between PD18

and PD24 with, this interval selected based on evidence that the pups' capabilities for object exploration are not firmly established before PD18 (Contreras et al., 2019) and that maturation of the hippocampus proceeds at least until PD24 (Travaglia et al., 2016; Donato et al., 2017). In two separate experiments, the developmental trajectory of the effects of early spatial experience on adult OPR performance and the role of sleep after infantile spatial experience were examined. For these experiments, two groups of pups were exposed to the "early spatial experience" manipulation during Early childhood (starting from PD25, n = 11 pups) and Adolescence (from PD48, n = 17 pups), respectively. A Sleep-deprivation group of pups (n = 13) was deprived of sleep after exposure to the early spatial experience during infancy. For these two separate experiments, also the Spatial-experience and No-experience control groups were newly formed to avoid multiple testing against the same reference groups. Furthermore, we aimed at enhancing statistical power as based on forgoing studies (Contreras et al., 2019), we expected increased response variability with the inclusion of the Early childhood and Adolescence groups. Two new sets of n = 12 rats and n = 12 rats were subjected to the same procedures as described for the Spatial-experience and No-experience groups of the main experiment, and it was assured (i) that the target effect of an enhanced OPR memory was replicated in these animals ($F(1, 20) = 5.437$, $P = 0.030$, for the difference in OPR memory between groups) and (ii) that the groups in OPR memory performance did not differed from the respective groups of the main experiments ($P > 0.642$, for all independent t-test comparisons on each minute of the OPR memory test). For statistical comparison the new sets were combined with the respective groups of the main experiments.

Experimental procedures and task

All procedures were performed between 7:00–16:00 h (i.e., the light phase). Animals received 5 sessions of 5 min handling on 5 (or 3, in 73 cases) consecutive days. For all groups (except the animals exposed to the early spatial experience at adolescence, i.e., PD48) the handling procedures included the dam in order to diminish potential stress. On each of the following 3 days, a 10-min habituation session was performed where the rats were allowed to freely explore the empty arena. In each session, they were introduced into the arena from a different side to support allocentric mapping. After the session, the rats were returned to the dam in the home cage.

On the day after the last habituation session, the rats of all groups were subjected to the early spatial experience. For the Spatial-experience group (and related control groups) this experience consisted of two 5-min visits of the arena in which two identical objects were placed. At the second of these visits one of the objects was moved to another place (Figure 1A). The 2 visits were separated by a 5-min break (for which the pup was brought to the home cage and dam) and, were repeated (with different objects and changes in spatial configuration) on 3 succeeding days (every other day). For the Non-spatial experience group, instead of displacing one of the objects, one of the objects was replaced by another during the second of the two visits. At each visit, the rat was introduced into the arena from a different side with the rat always facing the respective wall of the arena. Behavioral responses to the change in spatial vs. non-spatial configuration were comparable in terms of the time the animals spent exploring both objects ($F(1, 33) = 1.549$, $P = 0.222$, across the 4 days) and travelling in the arena ($F(1, 33) = 1.515$, $P = 0.227$). The No-experience control animals did not receive any experimental manipulation until adulthood.

At adulthood, all groups were tested on a classical Object-place recognition (OPR) task with the encoding phase and the retrieval phase separated by a 3-hour retention interval (during which the rats were moved to the home cage). Preparation for OPR testing included exactly the same procedures of 5-min handling (on 3 days), followed by 10-min sessions to habituate the animal to the empty arena (on 3 days). For the Long-term OPR control group, the encoding phase took place during infancy (PD24) and recognition was tested at adulthood (PD84), with handling and habituation procedures only preceding the infantile encoding phase. In the interval between the "early spatial experience" manipulation and adult OPR testing, the animals were kept in their home cages and weighted weekly, but otherwise remained without any experimental stimulation.

Apparatus and objects

For establishing the "early spatial/non-spatial experience" manipulation a quadratic dark grey open field was used (43 cm x 43 cm, height of walls 35 cm). Objects (height 10-18 cm) were glass bottles of different shapes, filled with water or sand of different colors. They had sufficient weight to ensure the rats could not displace them. To support allocentric spatial mapping, a number of distal cues were available: The North side of the arena was headed towards a white wall whereas the East and West sides were surrounded by a grey curtain. The South side of the arena faced a removable black curtain (which also served as the experimenter's entrance). Additional discrete distal cues were provided on the ceiling: a brown wood square (40 cm x 40 cm) located 120 cm above the open field and 36 cm below the ceiling. At two sides, a pink ball (10 cm diameter) and a light-brown cartoon box (25 cm x 25 cm x 10 cm), respectively, were attached to the curtains. Two fluorescent strip lights placed on the floor of the room provided

indirect light. White noise was presented at a constant intensity during all procedures, to mask any disturbing sounds.

OPR testing during adulthood took place in a similar but larger arena (77 cm x 77 cm, height of walls 37 cm), compared with that during early spatial experience. Objects were also larger (22-29 cm) and quite different from those during early experience. Distal cues were as described for the early experience, except for the Context-change group. The context of OPR testing for this group differed in several aspects: The experimenter was a different person (all experiments were conducted by women), the experimental room was different and all distal cues as well as texture (wrinkled vs smooth floor) differed. Objects and arena were cleaned thoroughly between trials with 70% ethanol solution.

Data collection and analysis

The rat's behavior was video-recorded during the visits of the early experience as well as during the encoding and retrieval phases of the OPR task and visually scored offline by an experienced experimenter using the ANY-Maze tracking software (Stoelting Europe, Dublin, Ireland). Exploration was defined by the rat directing its nose to the object and sniffing. Climbing on an object or sitting next to it without any signs of active exploration was not included.

On the OPR task, allocentric spatial memory was analysed using the object discrimination index which is the standard way to assess OPR memory in adult rats, and is defined by the formula: DI = [(exploration time for novel object-location – exploration time for familiar object-location) / (exploration time for novel object-location + exploration time for familiar object-location)]. Additionally, the total time of object exploration (across both objects) and the total distance travelled during encoding and retrieval phases were determined.

Sleep and sleep deprivation

The pups of the Spatial-experience group (as well as the pups of the Non-spatial-experience group) were returned to their home cage with their littermates and dam immediately after each second visit to the arena on PD18, PD20, PD22 and PD24. Assessment of (video-recorded) behavior assured that the pups spent a minimum of 44 min of the 90-min post-experience interval in a sleeping position, close to the dam and often fully covered by the dam's body. Visual inspections performed in a separate sample of 6 pups confirmed the presence of sleep (i.e., closed eyes and occasional suckling) during the times the pup was mostly covered by the dam's body. The rats of the Sleep-deprivation group were deprived from sleep during the 90-min post-experience interval applying a "gentle-handling" procedure. The procedure was initiated as soon as the litter huddled together or one of the pups showed signs of sleep (e.g., taking a sleep posture or closing its eyes), and consisted of tapping on the cage, gently shaking the cage, disturbing nest-building behavior and, to avoid huddling, separating the pups by placing them away from their littermates. During the post-experience intervals on PD18 and PD20 (preweaning), the dam was kept in a neighboring cage allowing that the pups to see and smell her.

c-Fos immunocytochemistry

After completing the retrieval phase of the OPR task, the rats were returned to their home cages for 90 min. Then, the animals were decapitated, and the brains were removed intact, frozen rapidly in metylbutane (Sigma-Aldrich, Taufkirchen, Germany), and stored at −40 °C. A 90-min delay after retrieval testing was used because c-Fos protein activity peaks with a latency of ~90 min after the event of interest (Bisler et al., 2002). The brains were coronally (30 µm) sectioned at -20 °C in a cryostat microtome (model HM 505-E, Microm International GmbH, Heidelberg,

Germany). The sections were mounted on gelatinized slides for c-Fos immunocytochemical analysis, and on non-gelatinized slides for additional cytochrome oxidase (COx) histochemistry (see below). We defined the regions of interest (ROIs) based on the literature about cortical, thalamic and hippocampal regions known to be involved in the formation of spatial and episodic memory formation (Eichenbaum, 2017), and determined the anatomically according to Paxinos and Watson's atlas (2005). ROIs and their distance (in mm) from bregma were: +3.24 mm for the prelimbic (PL), infralimbic (IL), and cingulate cortices (CG); -3.96 mm for the agranular (RSA) and granular retrosplenial cortex (RSG), for the parietal (PAR), perirhinal (PRC) and lateral entorhinal cortices (LEC); -1.80 mm for the thalamic nucleus reuniens (RE) and reticular thalamic nucleus (RTN); -3.96 mm for the hippocampal cornu ammonis 1 (CA1), cornu ammonis 3 (CA3) and dentate gyrus (DG) subfields.

Brain processing

For c-Fos immunocytochemistry, the sections were post-fixed in buffered 4% paraformaldehyde (0.1 M, pH 7.4) for 30 min and rinsed in phosphate-buffered saline (PBS, 0.01 M, pH 7.4). They were subsequently incubated for 15 min with 3% hydrogen peroxidase in PBS to remove endogenous peroxidase activity, and then washed twice in PBS. After blocking with PBS solution containing 10% Triton X-100 (PBS-T, Sigma, USA) and 3% bovine serum albumin for 30 min, sections were incubated with a rabbit polyclonal anti-c-Fos solution (1:10,000, sc-52, Santa Cruz Biotech, Santa Cruz, CA, USA) diluted in PBS-T for 24 h at 4°C in a humid chamber. Slides were then washed 3 times with PBS and incubated in a goat anti-rabbit biotinylated IgG secondary antibody (Thermo Scientific Pierce, Rockford, IL, USA; diluted 1:200 in incubating solution) for 2 h at room temperature. They were washed for another 3 times in PBS and then reacted with the avidin biotin peroxidase complex (Vectastain ABC Ultrasensitive Elite Kit,

Pierce, USA) for 1 h. After 2 more washes in PBS, the reaction was visualized by treating the sections for 3 min in a nickel-cobalt intensified diamino benzidine kit (Pierce, USA). The reaction was terminated by washing the sections twice in PBS. Slides were then dehydrated through a series of graded alcohols, cleared with xylene, and cover-slipped with Entellan (Merck, USA) for microscopic evaluation. All immunocytochemistry procedures included sections that served as controls where the primary antibody was not added. Slides containing sections of a specific brain region were stained at the same time. The experimenter performing the c-Fos and COx analyses was blind to the experimental conditions of the individual brains.

The total number of c-Fos positive nuclei was quantified in two alternate sections 30 µm apart. Quantification was performed by systematically sampling each of the regions selected using superimposed counting frames. Sizes of the counting frames ranged from 40,000 μm^2 (RE) to 360,000 μm^2 (PAR). The total area covered by these frames per region in each section was: 40,000 μm^2 in RE; 90,000 μm^2 in DG; 120,000 μm^2 in RTN and CA1; 180,000 μm^2 in RSA, RSG and CA3; 270,000 μm^2 in IL, PL, CG, PRC, and LEC; and 360,000 μm^2 in PAR. Cell counts were conducted using a microscope (Leica DFC490, Germany) coupled to a computer with software installed (Leica application suite, Germany). c-Fos positive nuclei were defined based on homogenous grey-black stained elements with a well-defined border. Finally, the mean count of two adjacent sections was calculated for each subject/brain and region.

Cytochrome oxidase (COx) histochemistry

As an estimate of basal metabolic rates in the ROIs, we additionally measured COx activity using quantitative histochemistry, as described elsewhere (Banqueri et al., 2017; Mendez et al., 2015). To quantify enzymatic activity and control staining variability across different baths, sets of tissue homogenate standards from rat brains were cut at different thicknesses (10, 30, 50 and

70 μm), and included in each COx staining bath together with the experimental brain sections. The batch standards of brain homogenate were previously analyzed by spectrophotometrical methods to measure mean COx activity and were used to generate a single regression equation between COx activity and the optical density of the experimental sections, as reference for the comparison of the different tissues (see below). The sections and standards were incubated for 5 min in 0.1 phosphate buffer with 10% (w/v) sucrose and 0.5 (v/v) glutaraldehyde, pH 7.6. Next, they were immersed in 3 batches of 0.1 M phosphate buffer with 10% (w/v) sucrose were given for 5 min each. Subsequently, 0.05 M Tris buffer, pH 7.6, with 275 mg/l cobalt chloride, 10% (w/v) sucrose, and 0.5 (v/v) dimethyl-sulfoxide was applied for 10 min. Then, sections and standards were incubated in a solution of 0.06 g cytochrome c, 0.016 g catalase, 40 g sucrose, 2 ml dimethyl-sulfoxide, and 0.4 g diaminobenzidine tetra-hydrochloride (Sigma-Aldrich, Madrid, Spain) in 800 ml of 0.1 M phosphate buffer at 37 °C for 1 h. The reaction was stopped by fixing the tissue in buffered formalin for 30 min at room temperature with 10% (w/v) sucrose and 4% (v/v) formalin. Finally, the slides were dehydrated, cleared with xylene, and cover-slipped with Entellan (Merck, Darmstadt, Germany).

The COx histochemical staining intensity was quantified by means of densitometric analysis, using a computer-assisted image analysis workstation (MCID, Interfocus ImagingLtd., Linton, England). The mean optical density (OD) of each region was measured in three alternate sections, 30 μm apart. In each section, four non-overlapping readings were taken, using a square-shaped sampling window adjusted for each region size. A total of twelve measurements were taken per region. Calibration of OD measures for COx activity units was performed using the stained homogenate standards for each staining batch. For each staining batch the software calculated a linear regression between optical density and COx activity, using the measured OD

attributed to each section. Average relative OD measured in each brain region was converted into COx activity units (1 unit: 1 μmol of cytochrome c oxidized/min/g tissue wet weight at 23 °C) using the calculated regression curve in each homogenate standard. The linear regression equations calculated to estimate COx activity from OD measures in the brain sections were also used to assess inter-batch variability which was < 1%.

Statistical analyses

All statistical analyses were performed using SPSS software (IBM, Armonk, NY, USA). Generally, results are reported as means ± SEM. A $P < 0.05$ was considered significant. Analyses of the discrimination index (DI) and related behavioral control measures (total exploration time and total distance travelled) at adult OPR testing were first performed for the entire 5-min interval of the retrieval phase, and then focused on the first min of this interval as it is known to most sensitively reflect memory (Winters et al., 2004). Statistical outliers were defined by a DI in the 1st min of the retrieval phase exceeding ±1.5 times the interquartile range (which correspond to the difference between the first and third quartile) (Field, 2009), and excluded from analyses (1 case each in the Spatial-experience and Non-spatial experience group, 2 cases in the Spatial-experience replication group). For the main experiment, a global analysis of variance (ANOVA) was performed with a Group factor representing the experimental groups (Spatial-experience, Non-spatial experience, No-experience, Long-term OPR, Context-change), and a repeated-measures Minute factor representing the 1st to 5th minute of the retrieval phase. The Huynh-Feldt correction was applied when the sphericity assumption was violated. To specify the significant Group x Minute interaction ($F(10.269, 182.269) = 0.357$, $P = 0.011$) from this analysis, subsequent ANOVA were performed on subsets of groups. Effects of age at spatial experience and sleep deprivation after infantile experience were tested in separate ANOVA

including the Early childhood and Adolescence groups and the Sleep deprivation group, respectively, in addition to the newly formed infantile Spatial-experience and No-experience groups. Early childhood and Adolescence groups were combined in these analyses to rule out potential biases resulting from unequal group sizes. Significant ANOVA main and interaction effects were followed by post-hoc pairwise *t*-tests (two-sided) and, in case of specific hypothesis testing, by planned contrasts. One sample *t*-test was used to test whether DI values differed from chance level (zero).

c-Fos and COx activity values were first analysed by a global ANOVA including a Group factor (Spatial-experience, Non-spatial experience, No-experience, Long-term OPR, Context-change and Sleep-deprivation) and repeated measures Areas factor (PL, IL, CG, RSA, RSG, PAR, PRC, LEC, RE, RTN, CA1, CA3, DG). Significant Group x Areas interactions (Huynh-Feldt corrected) were followed by one-way ANOVAs including a Group factor (Spatial-experience, Non-spatial experience and No-experience, or Spatial-experience, Context-change, and Long-term OPR; $n = 6$ for each group, but $n = 5$ for Context-Change group), which were performed separately for each area. Significances were followed by post-hoc pairwise *t*-tests (two-sided). For an exploratory functional connectivity analysis, Pearson correlation coefficients were calculated for c-Fos activity between all pairs of areas. Connectivity graphs were subsequently constructed using both c-Fos quantifications and correlation coefficients. The Igraph package (v1.2.4.2) in R (RStudio, Boston, MA, USA) was used to visualize the networks.

Results

Infantile spatial experience enhances OPR performance at adulthood.

At testing in adulthood, only the rats with infantile spatial experience showed robust capabilities to form persisting spatial memory on the OPR task. Retrieval performance of this group was significantly higher than in both the Non-spatial experience and the No-experience control groups ($F(4.944, 121.135) = 2.989$, $P = 0.014$ for Group × Minute ANOVA interaction). The enhancement was largest during the first minute of the retrieval phase which is typically most sensitive to the memory effect (Winter et al., 2004) ($F(2, 51) = 4.464$, $P = 0.017$, for main effect of Group, see Figure 1B for results from pairwise statistical comparisons; Figure S1A shows control parameters of total exploration time and distance travelled during retrieval). In fact, during this first minute of adulthood OPR testing only the Spatial-experience group exhibited consistent preference for the displaced object, and expressed it throughout the 5-min retrieval phase ($t(16) = 8.323$, 5.711, 4.326, 3.579 and 3.894, all $P < 0.003$ for comparisons with chance level performance per minute, Figure S2). Rats with Non-spatial experience during infancy did not express any significant spatial memory throughout the test period (all $t(16) > 0.423$ and $P > 0.074$), and the No-experience group only transiently expressed memory (2^{nd} and 3^{rd} min of retrieval phase; $t(17) = 2.147$ and 2.456, $P < 0.046$), the latter finding confirming previous studies testing OPR memory in adult rats using the same 3-hour delay (Contreras et al., 2019). These results demonstrate that discrete and short-lasting spatial experience during infancy can strongly enhance the capability to form persistent spatial representations in adulthood.

We asked whether the enhanced spatial memory capabilities in the Spatial-experience group were perhaps an immediate direct consequence of an episodic memory that was formed for the spatial experience during infancy and persisted into adulthood. To answer this question, we

tested rats on an OPR task with the encoding phase performed during infancy (PD24) and the retrieval phase occurring only later, in adulthood (PD84, **Long-term OPR** group). At this remote retrieval test, the rats did not exhibit significant spatial memory (as measured by preferential exploration of the displaced object) at any minute of the retrieval phase (Figure 1C). In line with other studies (Sawangjit et al., 2018; Guskjolen, et al., 2018; Westbrook et al., 2014), this result indicates that the rats do not retain an episodic representation of the spatial configuration over such a long time, thus excluding a persisting episodic memory as a cause for the Spatial-experience group's enhanced adult spatial performance. Curiously, exploring the whole 5-min test phase revealed that, starting from the 2nd minute, the rats displayed negative discrimination ratios, i.e., preferential exploration of the stationary object (all $t(11) > -3.195$, $P < 0.027$) suggesting that a rudimentary form of memory for the original infantile experience is preserved into adulthood (Laham et al., 2021; Contreras et al., 2019) (Figure S3 for discrimination ratio across minutes and Figure S1B for control parameters during retrieval).

We hypothesized that infantile spatial experience improved adult spatial memory capabilities via inducing a more generalized schema-like representations which in theory are context-independent (Sekeres et al., 2018). Against this backdrop, we examined whether context-independency is likewise a feature characterizing schema memories induced during infancy. To this end, we compared the Spatial-experience rats which performed the OPR task at adulthood in a very similar context as that used for inducing spatial experience during infancy (same experimenter, same distal cues, larger arena size) with a control group of Spatial-experience rats which performed the OPR task at adulthood in an entirely different context (**Context-change** group; Figure 1C). To our surprise, did the Context-change group not profit from the infantile spatial experience ($F(1, 27) = 7.490$, $P = 0.011$, for the difference between groups). In fact, the

Context-change rats did not perform above chance level at any minute of the OPR retrieval phase at adulthood (all $t(11) > -0.759$ and $P > 0.160$, Figure S3). Fittingly, in two recent studies, the recovery of an infantile memory for an aversive event during early adulthood was likewise found to be context-specific (Guskjolen et al., 2018; Travaglia et al., 2016), which in combination with the present findings supports the notion that the schema memories induced during infancy can also integrate contextual elements of the infantile experience (Stark et al., 2018; Ghosh and Gilboa, 2014).

c-Fos activity at OPR testing during adulthood in cortex, thalamus and hippocampus.

Based on studies of adults, the transformation of episodic into schema-like memories is thought to be mediated through a dialogue between the hippocampus and mainly cortical networks, whereby the hippocampus initially binds the distributed representations of an experience into a coherent episodic memory representation (Zhou et al., 2021; Klinzing et al., 2019; Sekeres et al., 2018; Frankland and Bontempi, 2005). Repeated reactivations of the neuronal representation support a gradual redistribution of the representation towards cortical networks eventually storing an abstracted schema version of the memory. It is in this context that we wondered to what extent the rats that experienced the spatial configuration change during infancy relied on neocortical and hippocampal networks when tested on the OPR task in adulthood. We examined expression of the activity-regulated gene c-Fos to map brain activity during adult OPR retrieval testing with a focus on hippocampal structures (CA1, CA3, dentate gyrus) and a thalamo-cortical system of regions well-known to contribute to the transformation of spatial episodic memory (Eichenbaum, 2017), and which essentially comprises the medial prefrontal cortex (mPFC, including prelimbic, infralimbic and cingulate cortices), the granular

retrosplenial cortex (RSG), the parietal cortex (PAR), the perirhinal cortex (PRC), and - as structures essential to connecting hippocampal and cortical systems - the thalamic nucleus reunions (RE) and the lateral entorhinal cortex (LEC) (Figure 2A). Remarkably, this analysis did not show any difference in c-Fos activity between the Spatial-experience group and the control groups of Non-spatial experience and No-experience in any of the hippocampal regions (all $F(2, 17) < 3.258$, $P > 0.067$). But, there were major differences in thalamic and cortical areas ($F(45.801, 274.806) = 5.518$, $P = 0.003$ for global ANOVA Group x Area interaction, see Figure 2B for pairwise comparisons). The rats with spatial experience during infancy, after adult OPR retrieval, displayed enhanced c-Fos activity in mPFC, specifically in the prelimbic cortex (PL) ($F(2, 17) = 5.952$, $P = 0.013$). Concurrently, c-Fos activity was consistently decreased in the PAR, PRC and also in the thalamic RE. Note, all of these changes were significant in comparison with both the Non-spatial experience and the No-experience group (Figure 2B). The pattern of increased c-Fos activity in the PL region of the mPFC but unchanged hippocampal activity characterizing the Spatial experience group was also confirmed in a comparison with retrieval-related c-Fos activity in the Context-change group (Figure S4). Functional connectivity analyses indicated that infantile spatial experience reduced interregional c-Fos coactivation to a few distinct connections, in comparison with the No-experience control group (Figure 3).

Together these results support the view that the enhancement in adult spatial learning capabilities after specific spatial experience during infancy is mediated by long-term schema representations that reside in cortical rather than hippocampal networks. The lack of differences in c-Fos expression in hippocampal networks well agrees with previous studies showing that the hippocampus is crucial for forming spatial memory, yet does not store long-lasting memories (Maviel et al., 2004; Gridchyn et al., 2020). In fact, consistent with the present findings, previous

studies have identified the medial prefrontal cortex and, particularly, its prelimbic subregion as a hub area mediating remote episodic memory recall (Chen et al., 2020; Kitamura et al., 2017; Wheeler et al., 2013; Tse et al., 2011; Jo et al., 2007; DeNardo et al., 2004). For remote recall, intracortical projections from this region may activate representations that, in the spatial domain, involve specific circuitry in the parietal and perirhinal cortices (Eichenbaum, 2017; Bicanski and Burgess, 2020). That the c-Fos response to OPR retrieval testing at adulthood in these more posterior cortices was consistently lower in the Spatial-experience group than in the two control groups might reflect a contraction and sharpening of the representations that can occur as a consequence of long-term experience (Polley et al., 2004). Interestingly, cytochrome oxidase (COx) activity, a trait marker of neuronal activity, was enhanced in the hippocampus (CA1, CA3) and entorhinal and perirhinal cortices following infantile experience, whether spatial or non-spatial in comparison with the No-experience control group, consistent with the idea that non-specific stimulation during early development generally fosters the function of hippocampal memory systems (Newcombe, 2019) (Figure S5). In conclusion, the advantage in spatial learning in our rats with spatial experience during infancy appears to originate from more efficient cortical long-term schema-like representations available for the regulation of retrieval behavior via the prelimbic mPFC.

Effects of early spatial experience on adult OPR performance depend on developmental age and post-experience sleep.

Is infancy a period when the brain is particularly capable of forming such supra-ordinate spatial long-term memory? The first years of life are characterized by distinct conditions of synaptic plasticity and the shaping of neuronal circuits mediating memory formation, and many

of these conditions, like a strongly elevated neurogenesis, particularly apply to the hippocampus, i.e., the structure centrally involved in the formation of spatial representations (Ramsaran et al., 2019; Newcombe, 2019; Alberini and Travaglia, 2017; Mullally and Maguire, 2014). In light of these pervasive alterations in hippocampal memory function during infancy, we tested the effects of early spatial experience on adult OPR performance at two further ages, i.e., in addition to a group of rats exposed to the spatial configuration change during infancy (PD18-PD24), in two other groups this exposure took place during **Early childhood** (PD25-PD31) and **Adolescence** (PD48-PD54), respectively. Note, these experiments also comprised new groups of rats for the No-experience condition and the Spatial-experience (during infancy) condition which replicated the effects of the main experiment (see Methods). OPR performance at adulthood testing was indistinguishable between the Early childhood and Adolescence groups ($t(26)$ = -0.053, -0.112, 0.033, 0.231 and -0.074, all $P > 0.819$ for pairwise comparison per minute), but was worse than that of the infantile Spatial-experience group ($F(1.883, 212) = 5.413$, $P = 0.007$ for Group x Minutes interaction, in an ANOVA with pooled Early childhood and Adolescence groups, Figure 4A). Importantly, the spatial experience benefitted adult OPR memory only in the pups exposed to this experience during infancy, whereas spatial experience occurring later during early childhood or adolescence left adult OPR performance entirely unchanged in comparisons with the No-experience control group ($P = 0.015$, for the planned contrast of the Spatial-experience group with all other groups; Figure 4A). Overall, the pattern of results agrees with our hypothesis that infancy is a period of particular sensitivity to spatial experiences and for taking them to build abstracted long-term memories (Bessières et al., 2020).

Sleep supports memory consolidation (Klinzing et al., 2019; Huber and Born, 2014; Stickgold, 2005). Sleep-dependent consolidation is thought of as an active systems consolidation

process that critically depends on hippocampal function (Sawangjit et al., 2018), and in which the repeated neuronal replay of newly encoded memories promotes the gradual transformation of memories into persistent and more abstract long-term memories (Klinzing et al., 2019). Sleep in human infants has indeed been found to promote the formation of abstracted memory for objects (Friedrich et al., 2015). We therefore asked whether the benefit from spatial experience during infancy for adult spatial learning capabilities depends on the occurrence of sleep after the infant experience. We compared the Spatial-experience group of rats which all showed normal extensive sleep after each visit of the arena with the change in spatial configuration during infancy, with a **Sleep-deprivation** group of rats which were subjected to a 90-min awake period following each of these visits. At adult OPR testing, performance of the Sleep-deprived rats was significantly worse than that of the Spatial-experience group ($t(38) = 3.042$, $P = 0.004$ for the first minute; $F(1, 38) = 4.933$, $P = 0.032$ for Group × Minute interaction), and did not differ from that of a No-experience control group ($t(23) = 0.831$, -0.282, -0.783, -1.424, and -1.105, all $P > 0.289$, for all comparisons; Figure 4B). In parallel, preventing sleep after the infantile spatial experience nullified the enhancement of retrieval-related c-Fos activity in the prelimbic medial prefrontal cortex characterizing the Spatial-experience group ($t(10) = 2.768$, $P = 0.020$, for pairwise comparison between groups). These results indicate that the beneficial effect of infant spatial experience on adult spatial learning requires sleep to occur after the infant experience.

Discussion

We present a novel approach that seeks to characterize the influence of discrete non-emotional spatial experience during infancy on spatial learning in adulthood. We find that a seemingly insignificant event, i.e., a change in the spatial configuration of two objects the rat is exposed to - in the absence of any rewarding or aversive stimulation - a few times during its infancy for overall no more than 20 min, strongly impacts learning behavior and related brain organisation during adulthood: This means at adulthood, the rats displayed enhanced capabilities to form persisting spatial memories, and this was specifically related to the use of spatial representational systems residing in cortical rather than hippocampal networks, whereby the prelimbic region of medial prefrontal cortex might represent a hub for regulating remote retrieval of these memories. Our findings, moreover, suggest that such discrete spatial events have the power to form memory for adulthood, only when experienced during infancy, but not during childhood or adolescence, and only when the experience during infancy is followed by sleep, underlining the paramount importance of infant sleep for forming lifelong memory (Friedrich et al., 2015; Gómez and Edgin, 2015).

Noteworthy, the enhancing influence of infant experience on adult spatial learning proved to be context-dependent, because the advantageous influence of infant experience on adult learning was observed only when the contexts at adulthood OPR testing and during infant experience were similar. This finding stands in contrast with concepts derived from research in adult brains, of systems memory consolidation assuming that the gradual corticalization of episodic representations leads to the formation of more abstracted memories that are independent of the original encoding context (Sekeres et al., 2018; Moscowich et al. 2016; Frankland and Bontempi, 2005). The enhanced context-dependency might be unique to memories abstracted

during infancy. An enhanced context-dependency of memory recall in infancy is well known (Robinson and Pascalis, 2004). At the same time, infants form more generalized memories neglecting detail, a tendency supported by sleep (Friedrich et al., 2015; Gómez and Edgin, 2015), and hallmarks of memory formation during early life have both been ascribed to the hippocampus being still immature and subjected to enhanced neurogenesis and, hence, less able to differentiate context and event (Ramsaran et al., 2019; Keresztes et al., 2018; Mullally and Maguire, 2014). These conditions in the hippocampus during infancy might as well favour the fast and sleep-dependent formation of long-lasting abstracted memory in cortical networks (Gómez and Edgin, 2015). In this framework, our findings demonstrate the powerful influence on adult behavior of discrete events occurring in seemingly irrelevant contextual conditions as part of the experience during an infant's everyday life.

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Autor contributions: M.I. and J.B. conceived the study; M.I., J.B. and M.P.C. designed the experiments; M.P.C. performed the experiments; M.P.C. and M.M. analyzed the data; M.I. and M.P.C performed statistical analyses with input from J.B; M.I. and J.B. wrote the manuscript.

Declaration of interest: Authors declare no competing interests.

Data availability statement

The materials generated in this study are available from the Lead Contact upon reasonable request.

Supplemental information consists of:

Supplemental Figure S1 – S3, related to Figure 1

Supplemental Figure S4 – S5, related to Figure 2

References

Akers, K. G. et al. (2014). Hippocampal neurogenesis regulates forgetting during adulthood and infancy. Science 344, 598-602.

Alberini, M. and Travaglia, A. (2017). Infantile amnesia: a critical period of learning to learn and remember. J. Neurosci. 37, 5783-5795.

Averbeck, B. B., Latham, P. E. and Pouget, A. (2006). Neural correlations, population coding and computation. Nat. Rev. Neurosci. 7, 358-366.

Banqueri, M., Méndez, M. and Arias, J. L. (2017). Spatial memory-related brain activity in normally reared and different maternal separation models in rats. Physiol. Behav. 181, 80-85.

Bessières, B., Travaglia, A., Mowery, T. M., Zhang, X. and Alberini, C. M. (2020). Early life experiences selectively mature learning and memory abilities. Nat. Commun. 11, 628.

Bicanski, A. and Burgess, N. (2020). Neuronal vector coding in spatial cognition. Nat Rev Neurosci. 21, 453-470.

Bisler, S. et al. (2002). Expression of c-Fos, ICER, Krox-24 and JunB in the whisker-to-barrel pathway of rats: time course of induction upon whisker stimulation by tactile exploration of an enriched environment. J. Chem. Neuroanat. 23, 187-198.

Chen, M. B., Jiang, X., Quake, S. R. and Südhof, T.C. (2020). Persistent transcriptional programmes are associated with remote memory. Nature 587, 437–442.

Contreras, M. P., Born, J. and Inostroza, M. (2019). The expression of allocentric object-place recognition memory during development. Behav. Brain Res. 372, 112013.

DeNardo, L. A. et al. (2019). Temporal evolution of cortical ensembles promoting remote memory retrieval. Nat. Neurosci. 22, 460-469.

Donato, F., Alberini, C. M., Amso, D., Dragoi, G., Dranovsky, A., and Newcombe, N. S. (2020). The Ontogeny of Hippocampus-Dependent Memories. J Neurosci. JN-SY-1651-20. Advance online publication. https://doi.org/10.1523/JNEUROSCI.1651-20.2020

Donato, F., Jacobsen, R. I., Moser, M. B. and Moser, E. I. (2017). Stellate cells drive maturation of the entorhinal-hippocampal circuit. Science 355, eaai8178.

Eichenbaum, H. (2017). Prefrontal–hippocampal interactions in episodic memory. Nat. Rev. Neurosci. 18, 547.

Farooq, U. and Dragoi, G. (2019). Emergence of preconfigured and plastic time-compressed sequences in early postnatal development. Science 363, 168-173.

Field, A. (2009). Discovering Statistics Using SPSS (SAGE Publications, London, ed. 3).

Frankland, P. W. and Bontempi, B. (2005). The organization of recent and remote memories. Nat. Rev. Neurosci. 6, 119-130.

Friedrich, M., Wilhelm, I., Born, J. and Friederici, A. D. (2015). Generalization of word meanings during infant sleep. Nat. Commun. 6, 6004.

Frostig, R. D. (2006). Functional organization and plasticity in the adult rat barrel cortex: moving out-of-the-box. Curr. Opin. Neurobiol. 16, 445-450.

Ghosh, V. E. and Gilboa, A. (2014). What is a memory schema? A historical perspective on current neuroscience literature. Neuropsychologia 53, 104-114.

Gómez, R. L. and Edgin, J. O. (2015). Sleep as a window into early neural development: Shifts in sleep-dependent learning effects across early childhood. Child. Dev. Perspect. 9, 183-189.

Gridchyn, I., Schoenenberger, P., O'Neill, J. and Csicsvari, J. (2020). Assembly-specific disruption of hippocampal replay leads to selective memory deficit. Neuron 106, 291-300.

Guskjolen, J. W. et al. (2018). Recovery of “lost” infant memories in mice. Curr. Biol. 28, 2283-2290.

Huber, R., and Born, J. (2014). Sleep, synaptic connectivity, and hippocampal memory during early development. Trends Cogn Sci, 18(3), 141–152.

Jo, Y. S., Park, E. H., Kim, I. H., Park, S. K., Kim, H., Kim, H. T., and Choi, J. S. (2007). The medial prefrontal cortex is involved in spatial memory retrieval under partial-cue conditions. J Neurosci. 27(49), 13567–13578.

Josselyn, S. A., and Frankland, P. W. (2012). Infantile amnesia: a neurogenic hypothesis. Learn Mem. 19(9), 423–433.

Keresztes, A., Ngo, C. T., Lindenberger, U., Werkle-Bergner, M. and Newcombe, N. S. (2018). Hippocampal maturation drives memory from generalization to specificity. Trends. Cogn. Sci. 22, 676-686.

Kitamura, T. et al. (2017). Engrams and circuits crucial for systems consolidation of a memory. Science 356, 73-78.

Klinzing, J. G., Niethard, N. and Born, J. (2019). Mechanisms of systems memory consolidation during sleep. Nat. Neurosci. 22, 1598-1610.

Laham, B. J., Diethorn, E. J., and Gould, E. (2021). Newborn mice form lasting CA2-dependent memories of their mothers. Cell Rep. 34(4), 108668.

Lupien, S. J., McEwen, B. S., Gunnar, M. R. and Heim, C. (2009). Effects of stress throughout the lifespan on the brain, behaviour and cognition. Nat. Rev. Neurosci. 10, 434-445.

Maviel, T. Durkin, T. P., Menzaghi, F. and Bontempi, B. (2004). Sites of neocortical reorganization critical for remote spatial memory. Science 305, 96-99.

Mendez, M., Arias, N., Uceda, S., and Arias, J. L. (2015). c-Fos expression correlates with performance on novel object and novel place recognition tests. Brain Res Bull. 117, 16–23.

Moscovitch, M., Cabeza, R., Winocur, G., and Nadel, L. (2016). Episodic Memory and Beyond: The Hippocampus and Neocortex in Transformation. Annu Rev Psychol. 67, 105–134.

Mullally, S. L. and Maguire, E. A. (2014). Learning to remember: the early ontogeny of episodic memory. Dev. Cogn. Neurosci. 9, 12-29.

Newcombe, N. S. (2019). Navigation and the developing brain. J. Exp. Biol. 222, jeb186460.

Paxinos, G. and Watson, C. (2005). The Rat Rrain in Stereotaxic Coordinates – The New Coronal Set (Elsevier Academic Press, London, ed. 5).

Polley, D. B., Kvasnák, E. and Frostig, R. D. (2004). Naturalistic experience transforms sensory maps in the adult cortex of caged animals. Nature 429, 67-71.

Ramsaran, A. I., Schlichting, M. L. and Frankland, P. W. (2019). The ontogeny of memory persistence and specificity. Dev. Cogn. Neurosci. 36, 100591.

Robinson, A. J. and Pascalis, O. (2004). Development of flexible visual recognition memory in human infants. Dev. Sci. 7, 527-533.

Sawangjit, A. et al. (2018). The hippocampus is crucial for forming non-hippocampal long-term memory during sleep. Nature 564, 109-113.

Sekeres, M. J., Winocur, G. and Moscovitch, M. (2018). The hippocampus and related neocortical structures in memory transformation. Neurosci. Lett. 680, 39-53.

Stark, S. M., Reagh, Z. M., Yassa, M. A. and Stark, C. E. (2018). What's in a context? Cautions, limitations, and potential paths forward. Neurosci. Lett. 680, 77-87.

Stickgold, R. (2005). Sleep-dependent memory consolidation. Nature 437, 1272-1278.

Travaglia, A., Bisaz, R., Sweet, E. S., Blitzer, R. D. and Alberini, C. M. (2016). Infantile amnesia reflects a developmental critical period for hippocampal learning. Nat. Neurosci. 19, 1225.

Tse, D. et al. (2011). Schema-dependent gene activation and memory encoding in neocortex. Science 333, 891-5.

Westbrook, R. S., Brennan, L. E. and Stanton, M. E. (2014). Ontogeny of object versus location recognition in the rat: acquisition and retention effects. Dev. Psychobiol. 56, 1492-1506.

Wheeler, A. L., Teixeira, C. M., Wang, A. H., Xiong, X., Kovacevic, N., Lerch, J. P., McIntosh, A. R., Parkinson, J., and Frankland, P. W. (2013). Identification of a functional connectome for long-term fear memory in mice. PLoS Comput Biol. 9(1), e1002853.

Winters, B. D., Forwood, S. E., Cowell, R. A., Saksida, L. M. and Bussey, T. J. (2004). Double dissociation between the effects of peri-postrhinal cortex and hippocampal lesions on tests of object recognition and spatial memory: heterogeneity of function within the temporal lobe. J. Neurosci. 24, 5901–5908.

Wong-Riley, M. T. (1989). Cytochrome oxidase: an endogenous metabolic marker for neuronal activity. Trends Neurosci. 12, 94-101.

Zhou, J., Jia, C., Montesinos-Cartagena, M., Gardner, M., Zong, W., and Schoenbaum, G. (2020). Evolving schema representations in orbitofrontal ensembles during learning. Nature 10.1038/s41586-020-03061-2.

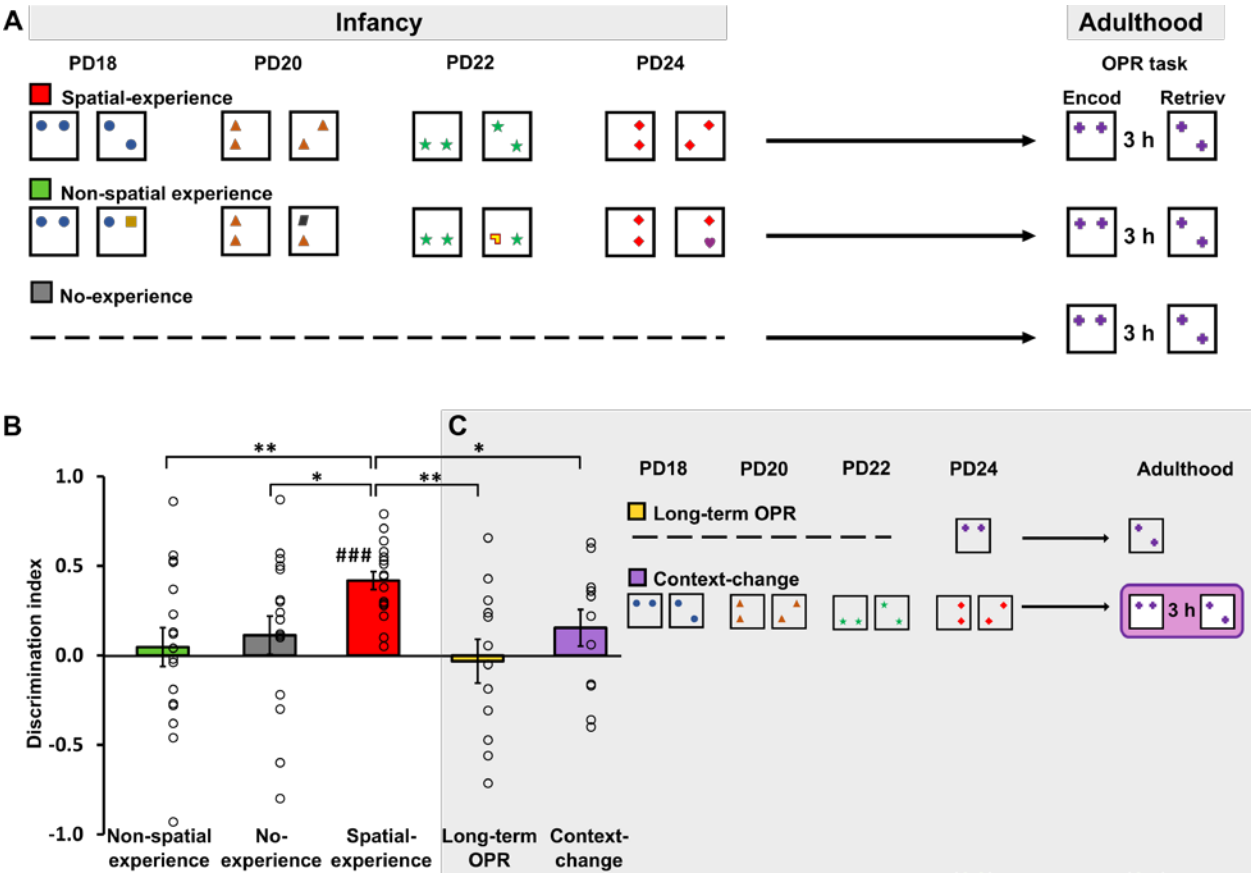


FIGURE 1. Effect of infantile spatial experience on adult OPR performance.

(A) General procedure: During infancy, pups of the Spatial-experience group (*n* = 17, red) were placed in an arena with two identical objects for 5 min and, after a 5-min break, re-entered to the arena but this time, one of the objects was displaced to a new location. Different spatial configurations and objects were used at the four arena visits, on PD18, 20, 22, and 24. For the Non-spatial experience group (*n* = 17 rats, green), instead of a change in object location, one of the objects was replaced by another, in the second 5-min period. The No-experience group (*n* = 18 rats, grey) had no arena visits during infancy. At adulthood (~PD80), all groups were tested on a classical object-place recognition (OPR) task with a 3-hour delay between encoding and retrieval testing.

(B) OPR memory (mean±SEM discrimination ratios during 1st min of retrieval phase, dot plots overlaid) at adulthood testing. Only rats with spatial experience during infancy displayed significant OPR memory.

(C) Grey shaded - Procedure of control experiments (right). For the Long-term OPR group (n = 12 rats, yellow), the OPR encoding phase took place during infancy (PD24) and retrieval testing at adulthood (PD84). Procedures for the Context-change group (n = 12 rats, purple) were the same as for the Spatial-experience group, except that OPR testing at adulthood was performed in a completely different context. Both Long-term OPR and Context-change groups did not show significant OPR memory (bottom). ### $P < 0.001$ for one-sample t-test against chance level; * $P < 0.05$ and ** $P < 0.01$ for pairwise comparisons (two-sided t-tests) between experimental groups. (See Figures S2 and S3 for discrimination ratios for entire 5-min retrieval phase).

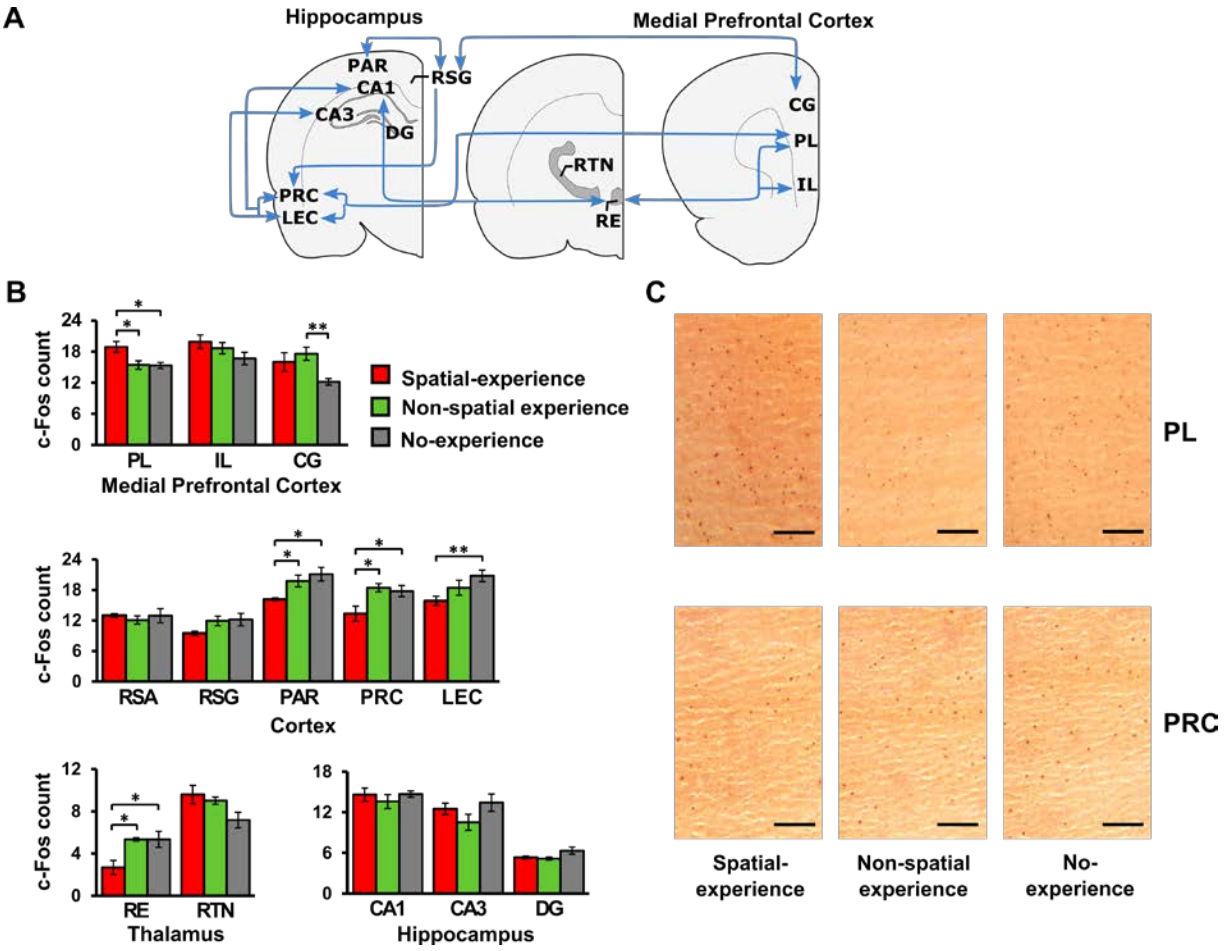


FIGURE 2. c-Fos activity in cortical, thalamic and hippocampal regions of interest.

(A) Schema of the selected regions of interest (ROI) contributing to memory formation in the hippocampus-dependent episodic memory system (modified from Eichenbaum, 2017).

(B) Mean±SEM counts of c-Fos^{+} cells in the Spatial-experience (red bars, n = 6), Non-spatial experience (green bars, n = 6), and No-experience groups (grey bars, n = 6) in (top) subregions of the medial prefrontal cortex, PL - prelimbic cortex, IL - infralimbic cortex, CG - cingulate cortex, (middle) the agranular retrosplenial (RSA), granular retrosplenial (RSG), parietal (PAR), perirhinal (PRC) and lateral entorhinal (LEC) cortices, (bottom left) in thalamic nuclei, RE - nucleus reuniens, RTN - reticular thalamic nucleus, and (bottom right) in hippocampal subfields,

CA1 - cornu ammonis 1, CA3 - cornu ammonis 3, DG - dentate gyrus. * $P < 0.05$ and ** $P < 0.01$ for pairwise comparisons (two-sided *t*-test) between experimental groups.

(C) Representative images of c-Fos staining selected for cell count analysis in PL and PRC (scale bar: 150 μm).

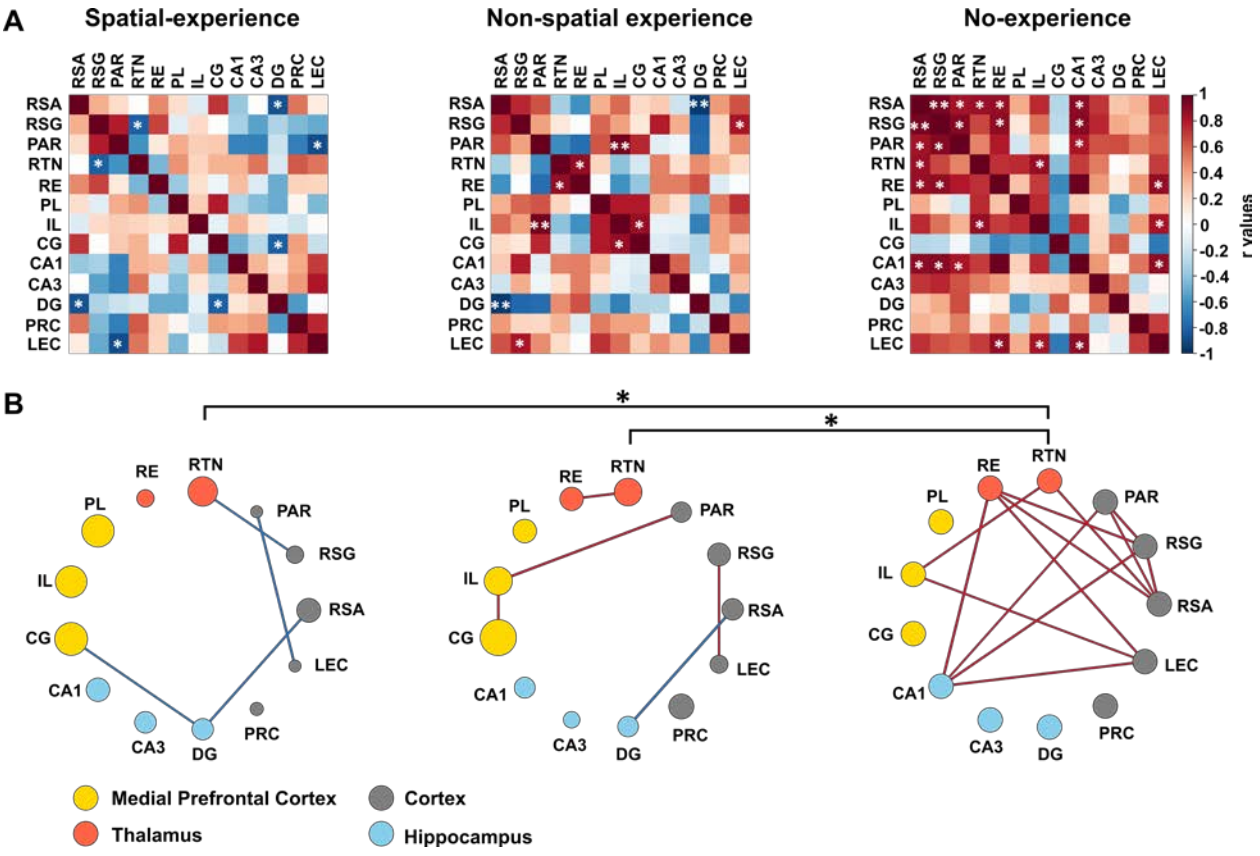


FIGURE 3. Network functional connectivity analysis based on c-Fos activity at OPR retrieval testing.

(A) Pearson correlation matrices showing inter-regional correlations for c-Fos activation in the Spatial-experience, Non-spatial experience, and No-experience groups (each group, $n = 6$). Axes represent the different brain areas. Correlation coefficients are color-coded (scale bars, right) and white asterisk (in the square) indicates significance (*$P < 0.05$ and ** $P < 0.01$, uncorrected for multiple comparisons).

(B) Network connectivity graphs depicting only significant ($P < 0.05$ and $P < 0.01$) *r*-values (red: positive, blue: negative). Different brain areas are color-grouped and node size for the Spatial-experience and Non-Spatial experience groups is proportional to the differential activation in comparison with the No-experience group (* $P < 0.05$, for the difference in number

of significant correlations between groups, for pairwise Fisher's exact test). Note, decrease in the number of significant inter-regional coactivations in the Spatial-experience and Non-spatial experience groups, in comparison with the No-experience control group, this decorrelation possibly reflecting a non-specific effect of infantile experience producing a general sharpening of arena-related representations that might more effectively process respective information (Frostig, 2006; Averbeck et al., 2006).

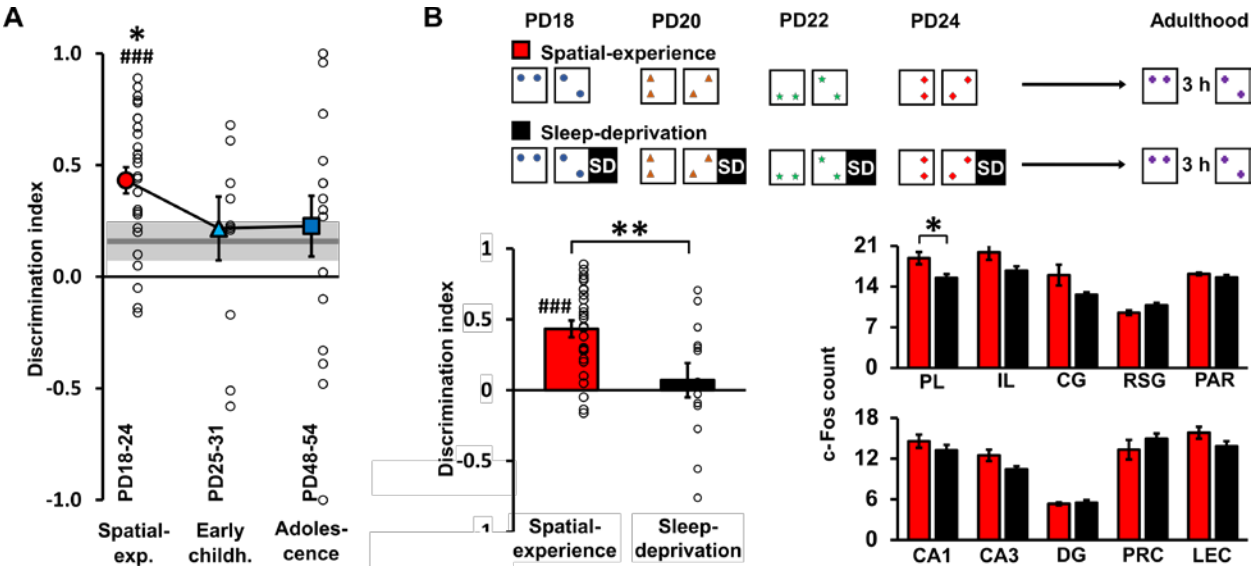


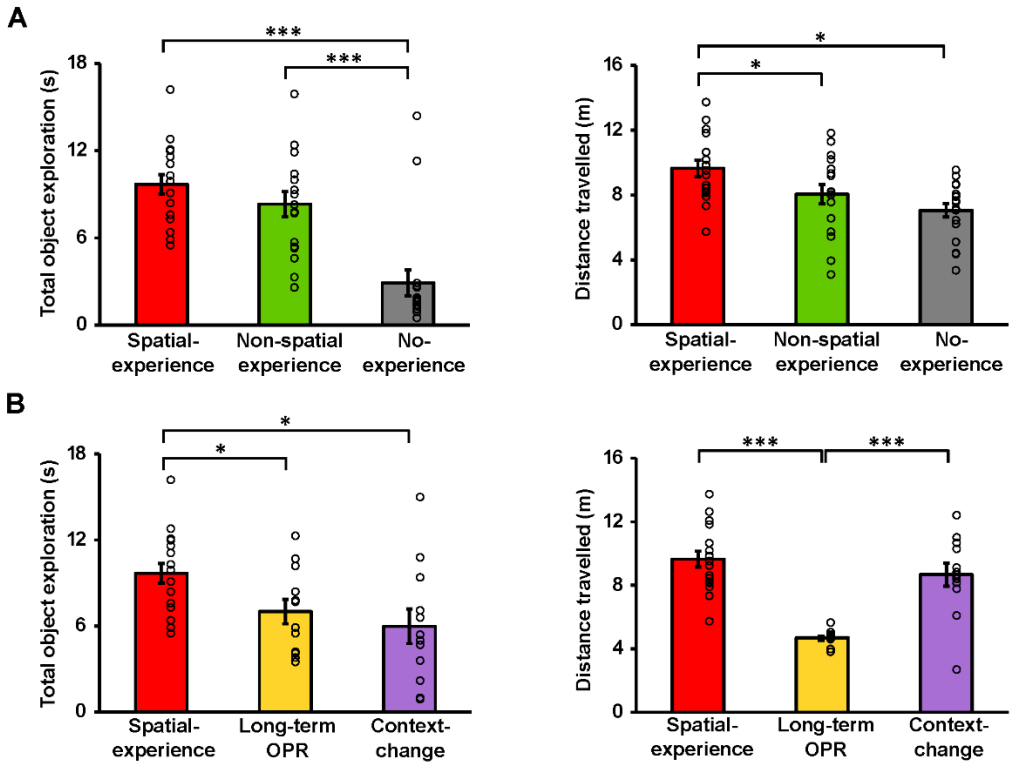
FIGURE 4. Developmental trajectory of the effects of spatial experience and the role of post-experience sleep.

(A) OPR memory (mean±SEM discrimination ratios during 1st min of retrieval phase, dot plots overlaid) at adulthood testing for the Spatial-experience group exposed to spatial experience during infancy (PD18-PD24, *n* = 27), for the Early childhood group (*n* = 11) and the Adolescence group (*n* = 17). OPR memory for the No-experience control group is indicated by horizontal line (± SEM grey shaded area). * *P* = 0.015 for planned contrast with all other groups, ### *P* < 0.001 for one-sampled *t*-test against chance level, $F(1, 56) = 1.318$, *P* = 0.256, for comparisons of Early childhood and Adolescence groups with the No-experience control. Note only the pups exposed to spatial experience between PD18-24 exhibit significant OPR memory that, in addition, is significantly stronger in comparison with all other groups.

(B) Top – Procedure: Pups either had undisturbed sleep (*n* = 27) or were deprived from sleep (SD) (*n* = 13) in the 90-min interval (black) after spatial experience. Bottom left – mean±SEM OPR memory for the two groups (dot plots overlaid). Bottom right - counts of c-Fos+ cells in

subgroups normal sleeping (n = 6 rats) and sleep deprived (n = 6 rats) animals in (top) subregions of the medial prefrontal cortex, PL - prelimbic cortex, IL - infralimbic cortex, and CG - cingulate cortex, and (bottom) in hippocampal subfields, CA1 - cornu ammonis 1, CA3 - cornu ammonis 3, and DG - dentate gyrus. * $P < 0.05$, ** $P < 0.01$ for pairwise comparisons (two-sided t-test) between experimental groups. ### $P < 0.001$ for one-sample t-test against chance level. Sleep deprivation nullified the enhancing effect of spatial experience during infancy on OPR performance during adulthood, along with the retrieval associated enhancement in c-Fos activity in the prelimbic PL region of the medial prefrontal cortex.

Supplementary figures

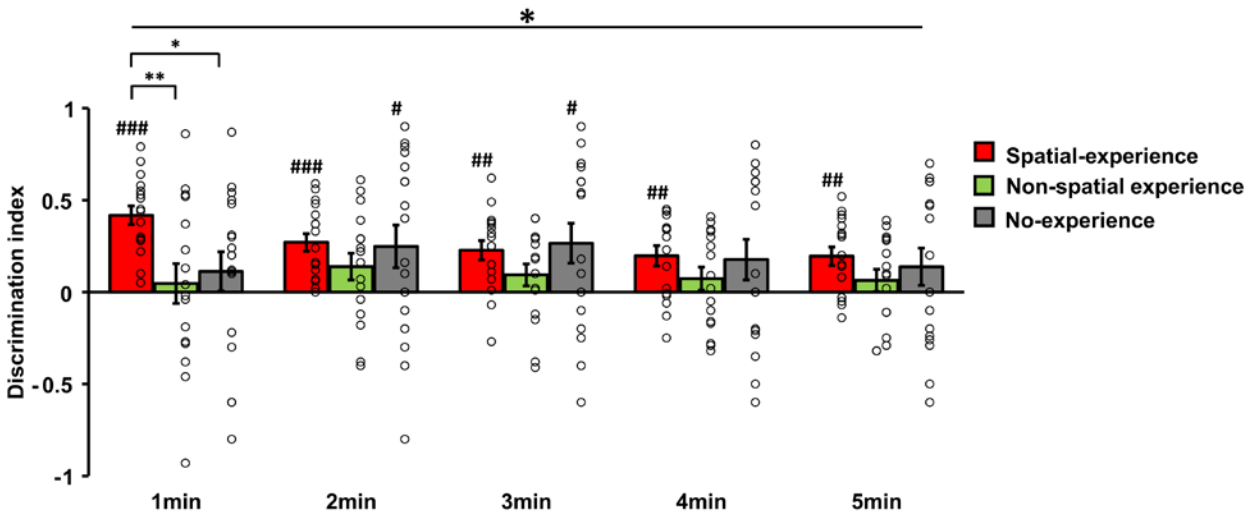


Supplemental Figure S1, related to Figure 1. Total exploration time and distance travelled during OPR performance.

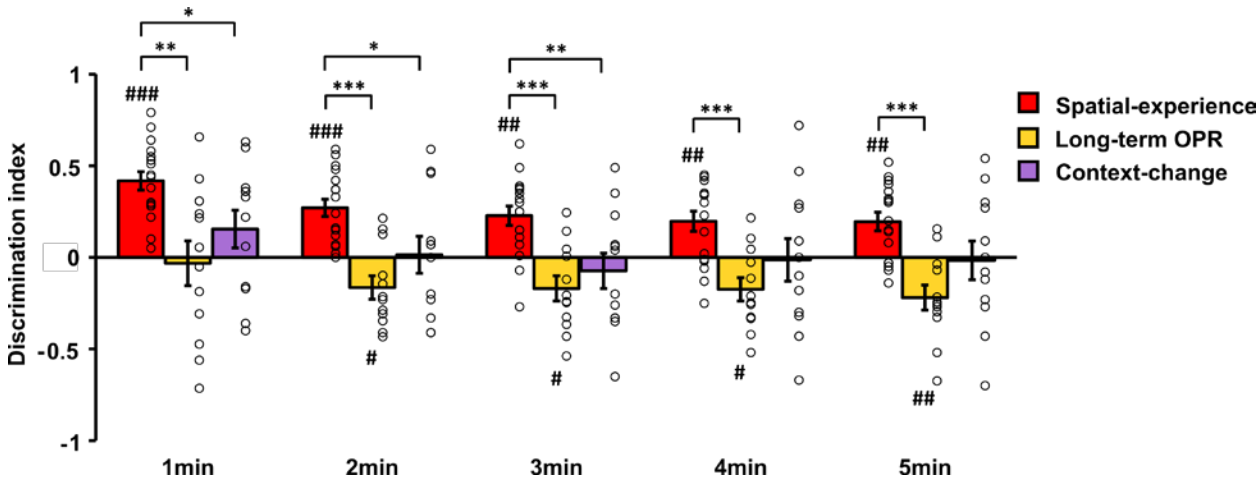
(A) Total object exploration time (in s) towards both objects (left) and total distance travelled (in m) during the retrieval phase (1st minute) for the Spatial-experience (red bars, *n* = 17), Non-spatial experience (green bars, *n* = 17) and No-experience groups (grey bars, *n* = 18).

(B) The same for the control experiments comparing the Spatial-experience group (red bars) with the Long-term OPR (*n* = 12 rats, yellow) and the Context-change group (*n* = 12 rats, purple). Means (±SEM) are indicated. * $P < 0.05$ and *** $P < 0.001$ for pairwise *t*-test (two-sided). Note, (in **A**) the No-experience control group shows less exploration and locomotion, likely reflecting a general effect of the lacking "arena-related" stimulation during infancy. Spatial-experience and

Non-spatial experience groups were closely comparable with respect to total exploration time. However, rats of the Spatial-experience group travelled a slightly greater distance than the rats of the Non-Spatial-experience group ($t(32) = -2.055$, $P = 0.048$), possibly reflecting general arousing effects on locomotion resulting from stimulation specifically of spatial systems during infancy. In **(B)** diminished total exploration time and distance travelled in the Long-term OPR group partly reflect the use of the smaller arena for retrieval testing (because for this group, the encoding phase took place already during infancy where testing was generally performed in a smaller arena). Moreover, compared with the Spatial-experience group, the Context change group shows reduced total exploration time for the objects ($t(27) = 2.864$, $P = 0.015$) possibly due to increased context exploration. We ruled out any contaminating effects of these control parameters on OPR memory, by performing analyses of covariance on the discrimination index using as covariate total exploration time and distance travelled, respectively. The memory effects were confirmed in all cases: i.e., OPR memory was distinctly stronger in the Spatial-experience group in comparison with the Non-spatial experience and the No-experience group ($P = 0.035$), the Long-term OPR ($P = 0.042$) and the Context-change groups ($P = 0.034$), independently of total exploration time or distance travelled (covariates in all cases remained non-significant $P > 0.409$).

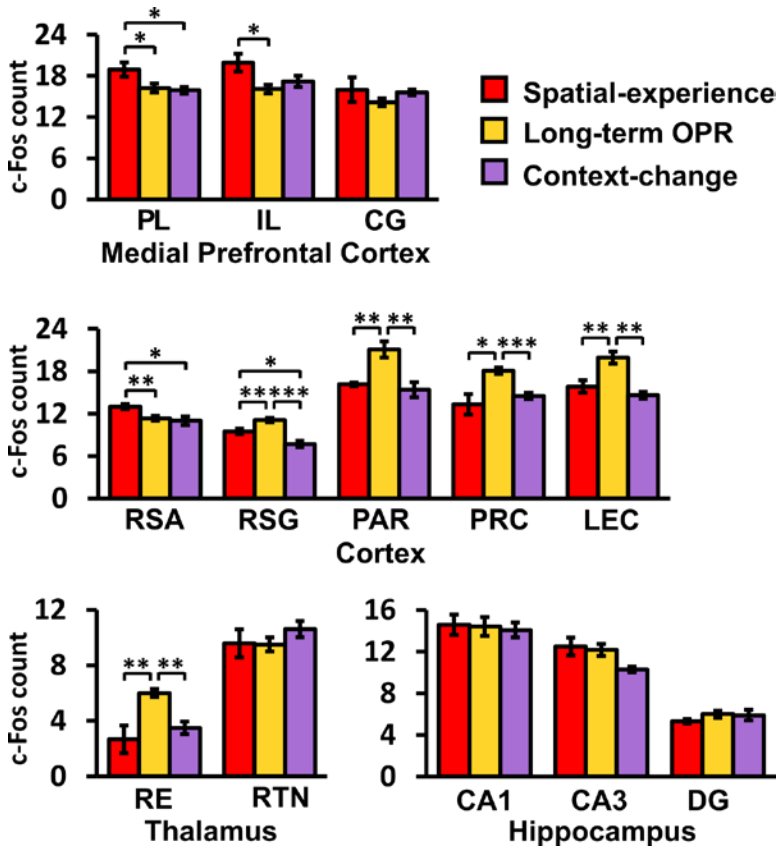


Supplemental Figure S2, related to Figure 1. Adult OPR performance for the entire 5-minute retrieval phase. Mean±SEM discrimination ratios separately during the first 1, 2, 3, 4 and entire 5 min of the retrieval phase (dot plots overlaid) at adulthood OPR testing, for the Spatial-experience (red bars, n = 17), Non-spatial experience (green bars, n = 17), and No-experience (grey bars, n = 18) groups. # $P < 0.05$, ## $P < 0.01$ and ### $P < 0.001$, for one-sample t-test against chance level; * $P < 0.05$ and ** $P < 0.01$ for pairwise comparisons (two-sided t-tests) between experimental groups. Horizontal line: $P < 0.05$ for Group x Minute ANOVA interaction. Note, enhanced OPR memory for the Spatial-experience group with, greatest difference between groups in the first min.



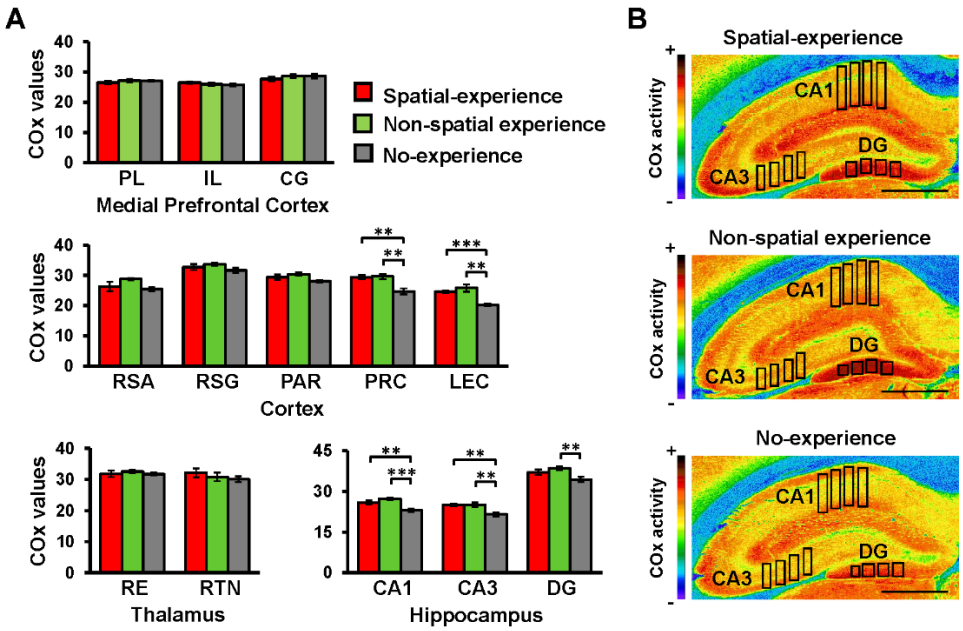
Supplemental Figure S3, related to Figure 1. OPR memory for the Long-term OPR and Context-change control groups. Mean±SEM discrimination ratios for the Long-term OPR (*n* = 12 rats, yellow bars) and the Context-change (*n* = 12 rats, purple bars) control groups shown separately during the first 1, 2, 3, 4 and entire 5 min of the retrieval phase, in comparison with the Spatial-experience group (*n*= 17; red bars; dot plots overlaid). For the Long-term OPR control group, the OPR encoding phase took place during infancy (PD24) and retrieval testing at adulthood (PD84). Procedures for the Context-change group were the same as for the Spatial-experience group, except that OPR testing at adulthood was performed in a completely different context. # $P < 0.05$, ## $P < 0.01$ and ### $P < 0.001$ for one-sample *t*-test against chance level. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$, for pairwise comparisons (two-sided *t*-tests) between experimental groups. ($F(1, 27) = 7.490$, $P = 0.011$, and $F(1, 27) = 31.943$, $P = 0.001$, for group main effect in ANOVA comparing the Spatial-experience group with the Long-term OPR and Context-change groups, respectively). Note, starting from the 2nd minute, the Long-term OPR group displayed significant negative discrimination ratios suggesting the presence of a rudimentary form of a memory for the original infantile experience which, curiously, is

expressed in an “infantile” manner, namely as familiarity preference (for the stationary object) rather than as novelty preference (Contreras et al., 2019).



Supplemental Figure S4, related to Figure 2. Comparison of c-Fos activity between the Spatial-experience, Long-term OPR, and Context-change groups. Mean±SEM counts of c-Fos^{+} cells in the Spatial-experience (red bars, n = 6), Long-term OPR (yellow bars, n = 6), and Context-change groups (purple bars, n = 5) in (top) subregions of the medial prefrontal cortex, PL - prelimbic cortex, IL - infralimbic cortex, CG - cingulate cortex, (middle) the agranular retrosplenial (RSA), granular retrosplenial (RSG), parietal (PAR), perirhinal (PRC) and lateral entorhinal (LEC) cortices, (bottom left) in thalamic nuclei, RE - nucleus reuniens, RTN - reticular thalamic nucleus, and (bottom right) in hippocampal subfields, CA1 - cornu ammonis 1, CA3 - cornu ammonis 3, DG - dentate gyrus. * P < = 0.05, ** P < 0.01 and *** P < 0.001 for pairwise comparisons (t-test) between experimental groups. In all groups, c-Fos was determined 90 min after the retrieval phase. Note, in comparison with both Long-term OPR and Context-

change groups, the Spatial-experience group shows enhanced c-Fos activity in the PL region of the medial prefrontal cortex ($t(10) = 2.152$, $P = 0.057$ and $t(9) = 2.412$, $P = 0.039$ for pairwise *t*-test comparison (two-sided) respectively) with no differences in hippocampal regions between groups. The enhance c-Fos activity mainly in posterior cortical regions (including PAR, PRC, LEC) and thalamic RE in the Long-term OPR group might reflect residual memory of the OPR encoding phase.



Supplemental Figure S5, related to Figure 2. COx activity in cortical, thalamic and hippocampal regions of interest.

(A) Mean±SEM values of COx activity in the Spatial-experience (red bars, n = 6), Non-spatial experience (green bars, n = 6), and No-experience groups (grey bars, n = 6) in (top) subregions of the medial prefrontal cortex, PL - prelimbic cortex, IL - infralimbic cortex, CG - cingulate cortex, (middle) the agranular retrosplenial (RSA), granular retrosplenial (RSG), parietal (PAR), perirhinal (PRC) and lateral entorhinal (LEC) cortices, (bottom left) in thalamic nuclei, RE - nucleus reuniens, RTN - reticular thalamic nucleus, and (bottom right) in hippocampal subfields, CA1 - cornu ammonis 1, CA3 - cornu ammonis 3, DG - dentate gyrus. ** $P < 0.01$ and *** $P < 0.001$ for pairwise t-test (two-sided).

(B) Representative images of COx-staining from hippocampal CA1, CA3 and DG regions. Rectangles indicate measured areas (scale bar: 1 mm). COx activity reflects the cell's basic metabolic rate as a trait marker of its activity level (Wong-Rilley, 1989) and was measured based

on optical density estimates (see Methods). Groups with experience (spatial and non-spatial) during infancy showed an increase in COx activity in the PRC ($F(2, 17) = 10.126$, $P = 0.002$) and LEC ($F(2, 17) = 14.780$, $P = 0.001$) cortices as well as in the hippocampal CA1 ($F(2, 17) = 12.440$, $P = 0.001$), CA3 ($F(2, 17) = 8.018$, $P = 0.004$) and DG subfields ($F(2, 17) = 5.911$, $P = 0.013$; $F(17.976, 134.819) = 2.428$, $P = 0.002$ for global Group x Area interaction).

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