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The relevance of the dorsal and ventral hippocampus for social recognition memory in mice

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Abstract

The present study was designed to investigate the involvement of the dorsal and ventral hippocampus in the generation of social recognition memory in adult male mice. First, we studied c-Fos synthesis in both hippocampal subareas in response to two distinct social learning sessions, indirect and direct. Next, we used local bilateral lidocaine infusions to temporarily inhibit the activity of either the dorsal or ventral hippocampus during the learning session only, and tested the subjects' social recognition memory using long-term retrieval conditions. Our data showed that a direct social learning session causes a significant increase in the number of c-Fos positive nuclei in the ventral, but not in the dorsal hippocampus. However, infusion of lidocaine in the dorsal hippocampus blocked long-term social recognition memory based on direct stimulus animal exposure but failed to affect recognition memory if only the volatile fraction of the olfactory signature was presented (indirect). Similar results were obtained with the infusion of lidocaine in the ventral hippocampus. The data of the present study suggests that both the dorsal and ventral hippocampus are causally involved in early processing of the information essential for long-term social recognition memory in mice. We suggest that both hippocampal subareas process the acquired information linked to the stimulation of different sensory modalities to recognize the conspecific.

1. Introduction

Social recognition memory describes the ability of animals and humans to recognize conspecifics on the basis of familiarity and individual recognition (Camats Perna & Engelmann, 2017). In contrast to rats, group housed mice easily consolidate a long-term social memory for a given conspecific when directly exposed in a learning (sampling) and recognition/discrimination session (recall or choice) lasting > 24 h (Kogan, Frankland, & Silva, 2000; Noack et al., 2010). The ability of mice to consolidate long-term social recognition memory seems to depend upon protein synthesis and involves different stages during the consolidation phase (Engelmann, 2009; Richter, Wolf, & Engelmann, 2005; Wanisch & Wotjak, 2008). In this context, it was shown that sampling under direct exposure conditions between the experimental subject and a stimulus animal triggers the *de novo* synthesis of the proto-oncogene c-Fos in distinct brain areas thought to be linked to the processing of the memory-relevant acquired information (Ferguson, Aldag, Insel, & Young, 2001; Richter et al., 2005). Indeed, subsequent c-Fos analysis confirmed a stimulus exposure-induced activation of defined brain areas. Own studies (Engelmann, 2009; Richter et al., 2005; Wanisch, Wotjak, & Engelmann, 2008) and that of others (Chowdhury & Caroni, 2018) suggested that the induction of c-Fos synthesis in cell assemblies might be linked to the consolidation of recognition memory.

The hippocampus, a brain area involved in memory formation as well as consolidation, is known to show segregated functionality along its longitudinal axis. Anatomical connectivity and gene expression analyses support this functional dissociation within traditionally recognized cytoarchitecturally defined boundaries (Fanselow & Dong, 2010). Processing of animal-related information in the ventral part of the hippocampus was suggested as being essential for intact recognition memory in mice (Deng, Gu, Sui,

Guo, & Liang, 2019; Phillips, Robinson, & Pozzo-Miller, 2019). However, the protocol of these experiments involved (1) no direct interaction between the experimental subject and the stimulus animals and (2) the experimenters tested short-term, but not long-term memory (Deng et al., 2019; Phillips et al., 2019). Interestingly, previous studies showed that short-term social memory does not require an intact hippocampus (Bannerman, Lemaire, Beggs, Rawlins, & Iversen, 2001; Kogan et al., 2000; Squires, Peddle, Milway, & Harley, 2006) and that the stimuli processed during direct interaction differ from that when only the volatile fraction of the olfactory signature of the conspecific (restricted due to caging the stimulus animals) is accessible for the experimental subject (Noack, Murau, & Engelmann, 2015). The present study aimed to investigate in more detail (1) the relevance of intact hippocampal function for long-term social recognition memory in mice, discriminating between the dorsal and ventral subareas, and (2) the differences of hippocampal activation patterns and its relevance depending on whether the experimental subject had direct or indirect access to the social stimuli presented.

2. Material and Methods

2. 1. Animals

Experimental subjects were adult male C57BL/6JOlaHsd mice with an age of 9–16 weeks (originally obtained from Harlan-Winkelmann, Borchern, Germany; further bread and kept in Zentrales Tierlabor der Medizinischen Fakultät der Otto-von-Guericke-Universität Magdeburg, Germany). If not stated otherwise, the animals were housed in groups of five per cage (size: 20 x 37 x 15 cm) under standard laboratory conditions with a 12h:12h light-dark cycle (light on: 07:00) for at least one week before starting the experiments. Juvenile mice of the same strain, aged 25–35 days, served as social stimuli. As previous studies revealed, the sex of the stimulus animals did not significantly affect the social recognition memory performance of the experimental subjects (M. Engelmann, Hädicke, & Noack, 2011; Hädicke & Engelmann, 2013), thus we used juveniles of both sexes.

All experimental manipulations including perfusions (see below) and killing the animals that were used in behavioural studies only after the end of the experiments by cervical dislocation were approved by the Committee on Animal Health and Care of the local governmental body and performed in strict compliance with the EEC recommendations for the care and use of laboratory animals (2010/63/EU and und 2007/526/EG).

2. 2. Surgery and substance infusion

Surgery: A detailed description of the general procedure used for bilateral implantation of guide cannulas in mice is provided elsewhere (Noack et al., 2015). Briefly, the experimental subjects were fixed in a stereotaxic frame (TSE Systems GmbH, Bad Homburg, Germany) under anaesthesia (Ketavet®, Pfizer Pharmacia, Berlin, Germany, and Rompun®, Bayer Vital GmbH, Leverkusern, Germany in 0,9% sodium chloride solution “Braun Melsungen AG, Melsungen, Germany), and analgesia (0.05 mg/kg body weigth, Metacam® Boehringer Ingelheim Vetmedica, Ingelheim, Germany). The frame-fixed animals were

bilaterally implanted with two guide cannulas reaching either the dorsal (from Bregma: 2.0 mm posterior, 1.3 mm lateral, 1.0 mm ventral) or the ventral hippocampus (from Bregma: 3.0 mm posterior, 2.8 mm lateral, 2.5 mm ventral). Additionally, two anchoring screws were placed next to the guide cannulas. Cannulas and screws were attached to each other and to the skull with light curling dental cement (Vivadent Schaan, Liechtenstein). The experimental subjects were allowed to recover from surgery for at least 7 days before starting the treatment experiment.

Infusion: Under light isoflurane anaesthesia, simultaneous bilateral microinfusions of lidocaine (20 µg/µl diluted in artificial cerebrospinal fluid, aCSF [Harvard Apparatus, Holliston MA, U.S.A.]) or aCSF were performed in a volume of 0.5 µl in each hemisphere via injection cannulas connected to a microinfusion pump (CMA Microdialysis, Stockholm, Sweden). The infusion cannulas extended the guide cannulas ventrally by 1 mm. The dosage of lidocaine and the treatment schedule followed the protocol of Noack et al. (2015). In the used concentration lidocaine inhibits action potential generation and propagation by blocking voltage-gated sodium channels, with a presumed duration of the effect < 60 min (Boehnke & Rasmusson, 2001; Malpeli & Schiller, 1979; Tehovnik & Sommer, 1997).

2. 3. Social discrimination procedure

The social discrimination test has been described in detail in two previous papers (M. Engelmann et al., 2011; M. Engelmann, Wotjak, & Landgraf, 1995). For the present study, the originally group housed experimental subjects were separated in their testing cages two h before testing during the given experiment. Each experiment consisted of two sessions (4 min each) separated by a 24 h exposure interval (EI). During the first session (sampling), a conspecific juvenile (J1; stimulus animal) was presented to the experimental subject, allowing the adult to acquire the juvenile's "olfactory signature". Then the stimulus animal was removed and the experimental subjects were kept individually in their testing cages. During the second session (choice) J1 was re-exposed to the experimental subject together with a second, previously not encountered juvenile (J2). During sampling and choice sessions a trained observer unaware of the experimental subjects' treatment monitored the behaviour of the experimental subjects towards J1 and – in case of choice – J2 separately by pressing pre-set keys on a computer keyboard. The key pressing was registered by a computer program (EVENTLOG 1.0, written by Robert Hendersen, U.S.A.) storing a data log file on the computer. The data log file was later analysed using a custom made analysis program (WinRat 4.0; written by Patrick Wild, Magdeburg, Germany). The following parameters were recorded: (1) social investigatory behaviour towards J1 (and J2) which included sniffing, licking and grooming and (2) aggressive/sexual behaviour towards the J1 (and J2) which included chasing, biting, kicking and mounting behaviour. A significantly longer duration of social investigatory behaviour towards J2 versus J1 during choice was taken as evidence for an intact recognition memory (M. Engelmann et al., 2011; M. Engelmann et al., 1995).

2. 4. Volatile fraction cage

In addition to the presentation of the full olfactory signature of the stimulus animal(s), as described above, experimental subjects were also tested in the volatile fraction cage (VFC). In this case, both sessions from the social discrimination procedure were performed using the VFC, preventing direct contact between experimental subjects and stimuli throughout the test. The construction of the VFC and its use was described in detail previously (M. Engelmann et al., 2011; Noack et al., 2010). Briefly, in the VFC the stimulus animal(s) are enclosed in small boxes through which an airstream produced by computer fans transfers the volatile fraction of the olfactory signature of the stimulus animals into a compartment that contains the experimental subject. The VFC allows the acquisition of the volatile fraction of the olfactory signature of the stimulus animal(s) by the experimental subjects. Both the non-volatile fraction of the olfactory signature of the stimulus animal(s) and tactile stimulation by the conspecific(s) is barred by the construction of the VFC, but acoustic stimuli between experimental subject and stimulus animal(s) can be exchanged. Again, a trained observer unaware of the animals' treatment measured the duration spend by the experimental subjects sniffing into the direction of the air steam. The testing principle was similar to the social discrimination test described above (i.e. sampling and choice sessions were separated by a given retention interval).

1. *2. 5. c-Fos immunohistochemistry*
2. *2. 5. 1. Stimulation, perfusion and tissue processing*

If not stated otherwise, mice were separated 24 h prior to the exposure of the defined stimulus in type II cages. The stimulus exposure was thought to imitate sampling in the social discrimination procedure and therefore lasted 4 min and consisted of either a direct exposure to a stimulus animal (sampling-like juvenile exposure as described in the social discrimination procedure) or indirect exposure to a stimulus animal (sampling-like juvenile exposure using the VFC). The control experimental subjects underwent similar manipulations, however without the presence of the stimulus animal. They also were isolated in a novel cage 24 hours prior to the start of the experiment. For those that underwent indirect exposure, they were additionally placed 10 min in the VFC before the presentation during four min of the fan-driven stream of room air. Two hours later, the experimental subjects were deeply anaesthetized an i.p. injection (Ketavet®, Pfizer Pharmacia, Berlin, Germany, and Domitor®, Orion Corporation, Espoo, Finland in 0,9% sodium chloride solution "Braun Melsungen AG, Melsungen, Germany) and transcardially perfused with physiological saline containing 0.1% Heparine (Heparin-Natrium 25000 ratiopharm®, Merckle GmbH, Blaubeuren, Germany) followed by 4% formaldehyde diluted in 0.1 M phosphate buffer (PBS, pH 7.4) for 12 min. Brains were removed, post-fixed for 24 h in 4% formaldehyde-PBS at 4°C with gentle shaking. The brains were then transferred to a sucrose solution (1 M in 0.1 M NaH2PO4 buffer) where they underwent gentle shaking at 4°C until the solution had infiltrated into the whole brain (for two to three days) in order to cryoprotect the tissue. Finally, the brains were embedded in a tissue freezing medium for cryo-sectioning (Leica Biosystems, Nussloch, Germany) and shock-frozen in isopentane at − 40°C - − 60°C and stored at − 80°C until cryo-sectioning (Leica CM3050 S Reseach Cryostat, Leica Biosystems, Nussloch, Germany).

2. 5. 2. c-Fos staining

c-Fos staining was performed as described elsewhere (Richter et al., 2005). Briefly, to eliminate endogenous peroxidase, floating coronal sections (25 µm) were placed in 24 well-plates and incubated for 20 min with 1:1 Methanol:PBS 1% H_2O_2 solution. After PBS washes, the non-specific binding sites were blocked using 10% normal goat serum solution (in PBS, 0.3% triton X-100 and 0.1% sodium azide) for 30 min and incubated with a rabbit polyclonal antibody raised against a peptide mapping at the amino terminus of human c-Fos p62 (identical to the corresponding mouse sequence; c-Fos sc-52; Santa Cruz Biotechnology, USA) diluted at 1:5000 in normal goat serum solution for ~ 70 h at 4°C. The sections were washed and incubated for one hour with 0.2% bovine serum albumin (in PBS, 0,2% triton X-100 and 0,1% sodium azide) in order to block unspecific binding sites. Next, the brain slices were incubated with the secondary goat biotinylated antibody (Diluion used 1:2000, goat anti-rabbit IgG, Vector Laboratories, Inc., Burlingame, USA) in bovine serum albumin solution overnight at RT. The sections were washed and incubated for one hour in 0.2% bovine serum albumin (in PBS only), and treated over four hours with the avidin-biotin peroxidase complex solution before being washed again. A change of pH was achieved by a 10 min washing step with 50 mM Tris solution. The visualization of the antibody binding sites involved the reaction of the avidin-biotin complex (Vector Laboratories, Inc., Burlingame) and the development by 3, 3'-diaminobenzidine (DAB, Sigma-Aldrich, Steinheim, Germany) (0.05% in 50 mM Tris solution, 0.5 ml / well) and its reaction with H_2O_2 (0.3%, 25 µl / well, for approximately four min) to produce a brown colorimetric end product which allows the labelling of c-Fos positive cells (ABC-method described elsewhere (Laube et al. 2002)). This reaction was stopped via washing. The brain slices were transferred to tap water and placed on gelatinized slides and dried with room air for at least one hour. An ascending alcohol series (in this order each for five min: 70%, 85%, 96%, 100% ethanol twice, and then twice ten min incubations with xylene) was followed by the final step to place glass coverslips onto the slides with the polystyrene mountant for coversliping DePex. Pictures were taken using a bright field microscope (Axio Imager; Zeiss, Germany) and the number of Fos-immunoreactive nuclei in each area was determined with a computer software program (Zeiss AxioVision Rel. 4.8.). A trained observer, who was unaware of the animals' treatment, counted the immunoreactive cells (c-Fos positive cells) in each area. The delineation between the paraventricular nucleus from the thalamus (PVT), the suprachiasmatic nucleus of the hypothalamus (SCN) (~ − 0.46 from Bregma), the medial amygdala (~ − 1.22 from Bregma), the dorsal hippocampus (~ − 1.94 from Bregma) and the ventral hippocampus (~ − 3.28 from Bregma) was made according to the stereotaxic mouse brain atlas by Franklin and Paxinos (1997). Three to five representative sections per animal were selected from approximately the same rostro-caudal level to analyse the listed brain areas. The total c-fos immunoreactive nuclei were quantified in the entire area from each section (including all subfields form the HC), except in the MeA in which a representative area of 0.1 mm^2 was chosen in each section and analysed.

2. 6. Experimental design of the behavioural experiments

All animals used in the study were tested for their long-term memory recognition performance in three sessions (without any treatment); the first session took place before surgery, the second, after surgery and the third, after all treatment sessions ended. The treatment experiments were performed in a double-blind, cross-over manner. The bilateral infusion of lidocaine and aCSF was performed directly before sampling with a recovery time of ~ 6 min between injection and the start of the respective session. Previous extensive testing confirmed that under the experimental conditions used, this duration of recovery is sufficient for an undisturbed behavioural testing (Noack et al., 2015).

2. 7. Statistics

Data were analysed using the statistical package GraphPad Prism 9.0.2 for Windows (GraphPad Software, Inc., La Jolla, CA 92037 USA). Data are shown as mean +/- SEM. Data obtained in the c-Fos analysis were tested using two-way ANOVA followed by Tukey HSD test. Investigation durations during choice were analysed using two-way ANOVA with repeated measures (factor juvenile) and paired Student's *t*-tests. Significance was accepted if $p < 0.05$.

3. Results

3. 1. c-Fos Immunohistochemistry

The number of c-Fos positive nuclei in both SCN and PVT did not differ under either treatment condition (data not shown). The number of c-Fos positive neurons in the MeA was increased in the animals exposed to a stimulus animal during a sampling-like exposure compared to unexposed animals (ANOVA $F(3,18) = 15.81$; $p < 0.001$), but did not differ between the treatment groups in the VFC (data not shown).

Under indirect conditions, using the VFC, no statistical differences in the number of c-Fos count was detected neither when comparing between hippocampal subareas (ANOVA: $F(1,16) = 4.220$; $p = 0.0567$) nor between stimuli presentation (ANOVA: $F(1,16) = 0.0122$; $p = 0.9134$). No differences were detected in the Tukey Post hoc analysis (Fig. 1B).

When the c-Fos data obtained from the different hippocampal subareas under direct stimulation conditions were statistically analysed, a significant effect of the hippocampal area (ANOVA: $F(1,20) = 14.66$; $p = 0.001$) as well as the stimuli presented (ANOVA: $F(1,20) = 7.818$; $p = 0.0112$) were revealed. Moreover, an interaction between these factors was also detected (ANOVA: $F(1,20) = 4.493$; $p = 0.0467$). Tukey post hoc analysis showed that a sampling-like stimulus animal exposure failed to significantly affect the number of c-Fos positive nuclei in the dorsal hippocampus ($p = 0.9630$) but it significantly increased the number of c-Fos positive nuclei count in the ventral hippocampus ($p = 0.0117$) (Fig. 1C). A more detailed analysis revealed that the c-Fos-labelling was primarily detected in the CA1 and subiculum areas of the ventral hippocampus (Fig. 1A).

3. 2. Behavioural data

Results of untreated experimental subjects before and after the injections/treatment experiments confirmed that not only before all manipulations, but also after surgery and at the end of all treatment experiments, the social recognition performance was intact and, thus, unaffected by the bilateral guide cannula implantation and the repeated treatment sessions (data not shown). After analysis of the histology, 12 animals had to be discarded because the infusion was judged to be outside of the respective brain area.

For the remaining animals (for histological slices showing representatively the correct targeting of the injection site see Fig. 2), analysis of the aggressive/sexual behaviour showed that there was no statistically significant difference in either the total duration or the number of bouts between the sessions in which aCSF and lidocaine was infused (data not shown).

Table 1 shows the investigation durations measured during sampling. There was no evidence that the treatment affected the experimental subjects' drive to investigate the conspecifics presented during the behavioural tests. As shown in supplementary Fig. 1 there was no evidence that the treatment affected the experimental subjects' drive to investigate the conspecifics presented during the behavioural tests under all treatment conditions during choice.

Table 1
Investigation durations (in seconds) during sampling (mean ± SEM). **A** shows the data collected in response to infusions in the dorsal hippocampus, **B** that of the data collected in response to infusions in the ventral hippocampus. VFC = volatile fraction cage. **A dorsal**

exposure	treatment	sampling	n
direct	aCSF	14.7 ± 1.7	20
direct	lidocaine	16.9 ± 1.8	20
VFC	aCSF	16.8 ± 2.1	19
VFC	lidocaine	20.5 ± 2.9	19

B ventral

exposure	treatment	sampling	n
direct	aCSF	10.5 ± 2.3	20
direct	lidocaine	9.6 ± 2.0	20
VFC	aCSF	17.2 ± 2.3	20
VFC	lidocaine	19.9 ± 2.6	20

3.2.1. Treatment of the dorsal hippocampus

The statistical analysis using two-way ANOVA of the investigation durations revealed significant effects of both treatment and juvenile for each hippocampus area (dorsal hippocampus: juvenile = $F(1, 75) = 14.27$, $p = 0.0003$; treatment = $F(3, 75) = 4.234$, $p = 0.0081$; ventral hippocampus: juvenile = $F(1, 76) = 11.70$, $p = 0.0010$, treatment = $F(3, 76) = 11.88$, $p < 0.0001$).

Figure 3A shows the impact of local treatment in the dorsal hippocampus during the acquisition session and tested 24 hours later, using both indirect exposure, using the VFC, and direct conditions. In the VFC, the infusion of aCSF into the dorsal hippocampus failed to affect the successful discrimination between the two stimuli presented during choice ($t = 3{,}141$, $df = 18$, $p = 0.0056$). Similar results were observed with lidocaine infusions, which also failed to affect social recognition ($t = 2.247$, $df = 18$, $p = 0.0374$).

When aCSF was infused into the dorsal hippocampus and subjects tested using direct exposure, the social recognition abilities of the experimental subjects were not affected as indicated by the significantly different investigation durations measured during choice ($t = 2.138$, $df = 19$; $p = 0.046$). In contrast, infusion of lidocaine abolished the different investigation durations under otherwise similar testing conditions and thereby indicated interference with memory performance ($t = 0.1060$, $df = 19$; $p = 0.917$).

3.2.2. Treatment of the ventral hippocampus

The treatment effects in the ventral hippocampus on the social recognition performance during direct exposure and in the VFC are illustrated in Fig. 3B. Bilateral aCSF infusion in the ventral hippocampus did not interfere with long-term social memory under direct exposure conditions ($t = 2.239$, $df = 19$; $p = 0.037$). In contrast, lidocaine infusion blocked long-term social recognition memory ($t = 0.8005$, $df = 19$; $p = 0.433$).

Both aCSF and lidocaine infusions in the ventral hippocampus produced an effect indicating an impaired discriminatory performance in the VFC; which, however, was just on the edge of reaching statistical significance for an unimpaired performance (aCSF: $t = 1{,}917$, $df = 19$, $p = 0{,}0704$; lidocaine $t = 1{,}773$, $df = 19$, $p = 0{,}0922$).

4. Discussion

The aim of this study was to investigate the relevance of the dorsal *versus* the ventral hippocampus for processing social stimuli needed for successful long-term social recognition memory formation. To do that, we first monitored the c-Fos synthesis in response to sampling-like juvenile exposure both directly and in the VFC with special reference to both hippocampal subareas. Further, we tested the impact of temporarily inhibiting the dorsal and ventral hippocampus during the sampling session of the social discrimination test and tested for long-term memory under direct conspecific exposure and indirect, using the VFC.

The immunohistochemical data obtained in the SCN and PVT suggest that the general physiological state was similar among the animals of all groups. Sampling-like juvenile exposure, but not exposure in the VFC triggered *de novo* c-Fos synthesis in the MeA. This is in line with previous observations using social discrimination learning (Richter et al., 2005) and suggests that also possible differences observed in other brain areas are likely due to the defined stimulus exposure.

We failed to observe an effect of the stimulus exposure on c-Fos-immunoreactive nuclei in the dorsal hippocampus (Fig. 1). This contrasts the findings of Ferguson et al. (2001), who reported an increased c-Fos staining in both the dentate gyrus and CA3 region of the dorsal hippocampus. We do not have an easy explanation for this discrepancy as methodological differences may not sufficiently explain the difference. However, also in our previous studies (Engelmann, 2009; Richter et al., 2005) we failed to observe a significant impact of sampling-like stimulus-animal exposure on the number of c-Fos positive neurons in the dorsal hippocampus (cf. Richter et al., 2005). Thus, discrepancies may originate in the difference in the stimulus animals used: castrated adult females (Ferguson et al., 2001) *versus* juveniles (as used in all our studies). Interestingly, an increased number of c-Fos positive neurons was detected in the ventral hippocampus in response to direct stimulus exposure only (Fig. 1) implying its selective activation by conspecific encounter. It is of note that the presentation of the volatile fraction of the olfactory signature in the VFC failed to affect the number of c-Fos positive nuclei in both parts of the hippocampus. Thus, the observed protein synthesis in the ventral hippocampus was triggered by stimuli that can be acquired by direct social interaction with the conspecific only, in particular taction, taste and the processing of the non-volatile fraction of the olfactory signature (Perna, Wotjak, Stork, & Engelmann, 2015). The latter is relevant as it was reported that the volatile rather than the non-volatile fraction of the olfactory signature is required for long-term social memory (Noack et al., 2010).

Studies with rats imply an increased social investigatory behaviour after cytotoxic lesion of the whole hippocampus (Bannerman et al., 2001). We failed to detect a similar effect in our experimental setting (see Table 1 & Fig. 3). This suggests that permanent lesions of this brain area induce effects different from acute, temporary lesions using lidocaine targeting distinct parts of the hippocampus. Other than in the case of temporarily lesioning the MeA with lidocaine (Noack et al., 2015), we also failed to find a significant impact of the infusion of lidocaine on sexual/aggressive behaviour monitored in parallel to social investigatory behaviour (unpublished data). We suggest, that the altered ability to recognize a conspecific is a specific effect induced by lidocaine infusion which is based on the drugs' ability to block

the electrical activity – and thus the acquisition and/or consolidation of social memory – in the temporarily targeted brain area.

If we extrapolate the area covered by the diffusion of the infused drug from the data obtained by protein synthesis inhibition caused by similar local infusions of an anisomycin solution 0.5 h after administration (~ 1.5 times of the estimated effective time window for the action of lidocaine) (Wanisch & Wotjak, 2008), our lidocaine infusion into the dorsal hippocampus is unlikely to have blocked the information transfer within the ventral hippocampus and *vice versa*. Infusions into the dorsal hippocampus are likely to have affected the activity not only of this part of the hippocampus, but also of the cortical areas above the injection site. Thus, in further studies we will investigate the relevance for information processing within the cortical areas adjacent to the ventral and dorsal hippocampus for long-term social recognition memory in the mouse.

Mono-modal stimulus exposure (VFC) just failed to reach statistical significance in animals infused with aCSF in the ventral hippocampus; a similar result of the statistical analysis was obtained in response to lidocaine infusion (Fig. 3). This may point towards a mild interfering effect of the infusion of aCSF in the ventral hippocampus *per se*. Unlike the multi-sensory modalities activated by direct stimulus animal exposure (Kummer et al., 2011; Strasser & Dixon, 1986), the “memory trace” induced in the VFC is likely to be more fragile and – therefore – is more sensitive to potentially interfering stimuli including a slightly altered local extracellular fluid composition. Indeed, among the different to-be-recognized stimuli, a directly exposed conspecific juvenile was the most robustly recognized stimulus tested in a retroactive interference study combined with isoflurane anaesthesia (Perna et al., 2015). When directly encountered, our results highlight the relevance of the hippocampus, both the dorsal and the ventral, during the early stages of social memory formation (Fig. 3A and 3B). Theoretically, it could be that lidocaine injections are affecting the novelty preference of the experimental subjects and thus subjects are unable to show recognition in the paradigm used in the present study. However, we think this is unlikely as the experimental subjects tested in the VFC showed preference towards the novel odours/stimuli during the choice session, thus indicating that novelty preference is intact in those animals injected with lidocaine in the same area. Those animals also showed intact social recognition memory, and thus novelty preference, when tested after receiving surgery and all the injections/treatments. Moreover, despite the expression of novelty preference involves cognitive function and recognition memory, response to novel events depends on the activation of midbrain reward system, including the substantia nigra and ventral tegmental area (Tapper & Molas, 2020), areas that are not affected in the tested experimental subjects.

Surprisingly, despite conspecific direct exposure did not lead to a significant c-Fos synthesis in the dorsal hippocampus, when this brain area was inactivated during acquisition it blocked long-term memory formation. In this study, c-Fos from all hippocampal fields was analysed together focusing on the differences between the dorsal and the ventral hippocampus, hence we could have missed subtle differences in specific hippocampal subfields which have been described to be relevant for the acquisition of other types of declarative memories (Roy et al., 2017). In future studies, we plan to further

investigate the involvement of the different hippocampal subfields in memory acquisition, especially during direct social interactions.

Barker and Warburton (2011) suggested that "… the hippocampus plays a role in recognition memory when such memory involves remembering that a particular stimulus occurred in a particular place or when the memory contains a temporal or object recency component". Ruediger, Spirig, Donato, and Caroni (2012) assigned "…a key function to ventral hippocampus in the early stages of complex trial-and-error learning". The results of the present study extend these hypotheses by suggesting that both the dorsal and ventral hippocampus are required for the acquisition and/or consolidation of items, if the items themselves stimulate different sensory modalities of the experimental subject. Given the experimental evidence that the dorsal *versus* ventral hippocampus are differently involved in processing memory relevant information, one might speculate that also for social recognition (although we observed in the present study similar effects in response to aCSF and lidocaine infusions in both hippocampal parts) the contribution of the dorsal hippocampus was different from that of the ventral hippocampus. We provide with Fig. 4a hypothesis that could explain the present and our previous findings in the context with the relevance of the dorsal and ventral hippocampus in social long-term memory in mice. Based on this hypothesis we aim to further unravel this functional difference and determine the contribution of the dorsal and the ventral hippocampus to long-term social recognition memory. Moreover, the results of the present study underline that in our social discrimination procedure during the direct exposure the experimental subjects acquired not only the juvenile's "olfactory signature", which involves volatile and non-volatile odours, but also non-odorant stimuli of the stimulus animals and thus may be better referred as "social signature".

Declarations

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Author's contribution:

JCP, RM and AM performed experiments. JCP and ME planned the experiments and wrote the manuscript.

Conflict of interest:

All authors declare no conflict of interest.

Ethics declaration:

All experimental manipulations – including housing and breeding – were approved by the Committee on Animal Health and Care of the local governmental body (Landesverwaltungsamt Sachsen-Anhalt, Referat

203) and performed in strict compliance with the EEC recommendations for the care and use of laboratory animals (2010/63/EU and und 2007/526/EG, see also Materials & Methods).

Submission declaration:

The work described here has not been published previously, that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere including electronically in the same form, in English or in any other language, without the written consent of the copyright-holder. Parts of the results were reported in poster and abstract form. The work published here is part of a PhD thesis (CP, J.: Distinction between dorsal and ventral hippocampus in processing social cues and the underlying mechanisms essential for social recognition memory in mice; available under http://dx.doi.org/10.25673/4616).

Consent for Publication

Not Applicable”

Data Availability Statement:

The data used in this study were generated by the authors and are available from the corresponding author upon reasonable request.

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Figures

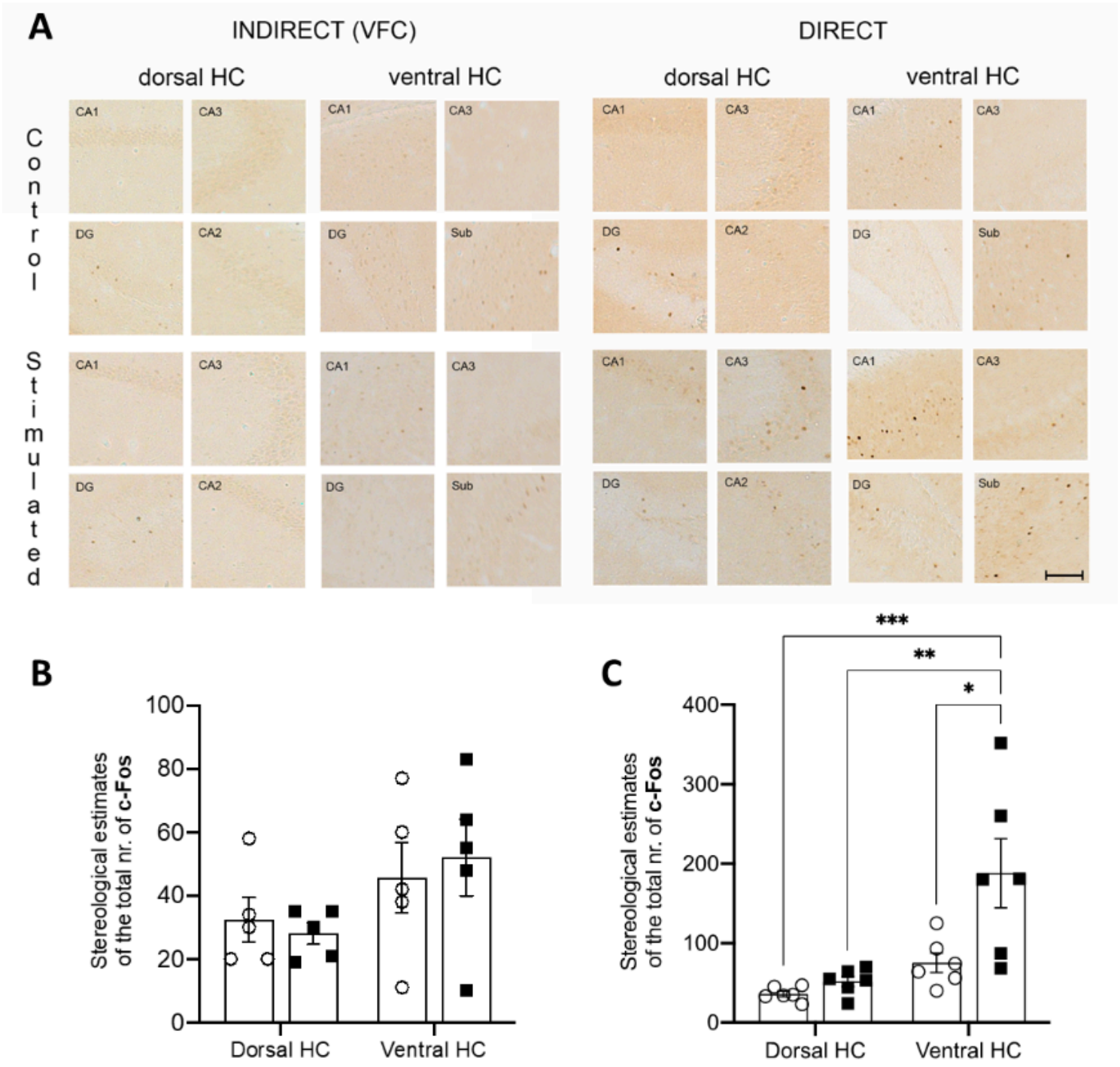


Figure 1

Results of the analysis of the c-Fos immunohistochemistry for the dorsal and ventral hippocampus in response to a 4-min sampling-like stimulus animal exposure either indirectly using the volatile fraction cage (VFC) or directly. **A**shows relevant parts of representative dorsal and ventral hippocampal coronal slices stained for c-Fos obtained from an unexposed control animal (control, white circles in **B & C**) and from an animal with direct or indirect (VFC) stimulus animal exposure (stimulated, black rectangles in **B & C**). CA1 = CA1 region of the hippocampus, CA2 = CA2 region of the hippocampus, CA3 = CA3 region of the ventral hippocampus, DG = dentate gyrus, Sub = subiculum; Scale bar in **A** = 100 µm. **B** Results of the

analysis of the c-Fos immunohistochemistry for the dorsal and ventral hippocampus (HC) in response to a 4-min sampling-like stimulus animal exposure in the volatile fraction cage (VFC). White symbols show individual data of animals exposed to an airstream in the VFC (control). Black symbols illustrate individual data of animals exposed to an airstream in the VFC with the presence of a stimulus animal (stimulated). Means ± SEM are represented. **C**Results of the analysis of the c-Fos immunohistochemistry for the dorsal and ventral hippocampus (HC) in response to a direct 4-min sampling-like stimulus animal exposure. White symbols show individual data of unstimulated experimental subjects. Black symbols illustrate individual data of animals exposed directly to a stimulus animal (stimulated). Means ± SEM are represented. Results of two-way ANOVA Tukey post-hoc test are shown, *p<0.05, **p<0.01,***p<0.001 ; n=6 per group.

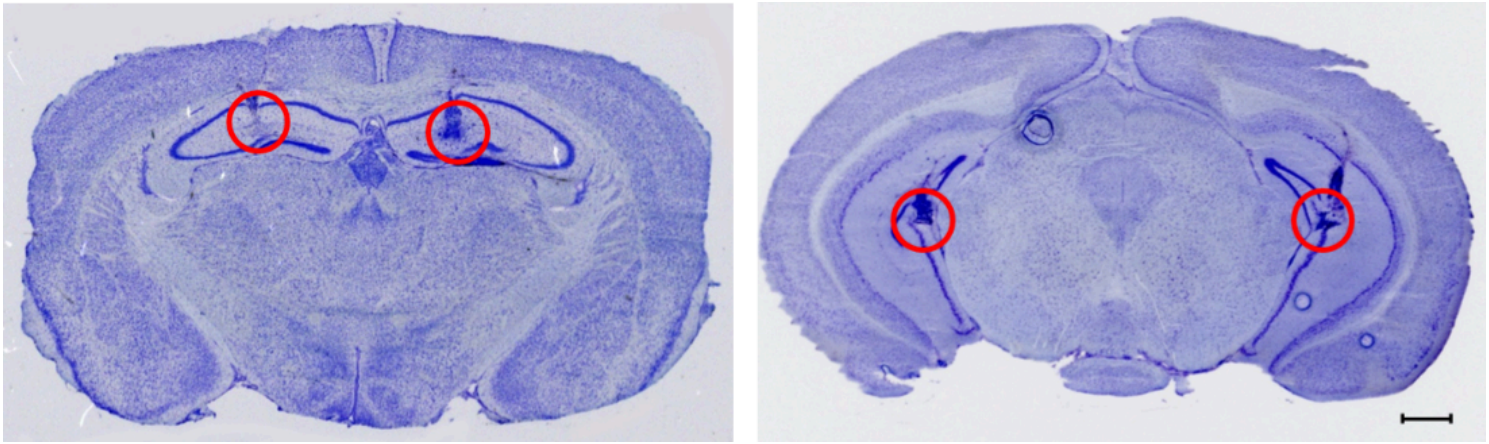


Figure 2

Photomicrographs showing representative histological slices with the reconstructed bilateral localisation of the injection site (red circle) of **A** the dorsal and **B** the ventral hippocampus from animals used in the present study. Scale bar for A and B in B = 100 µm.

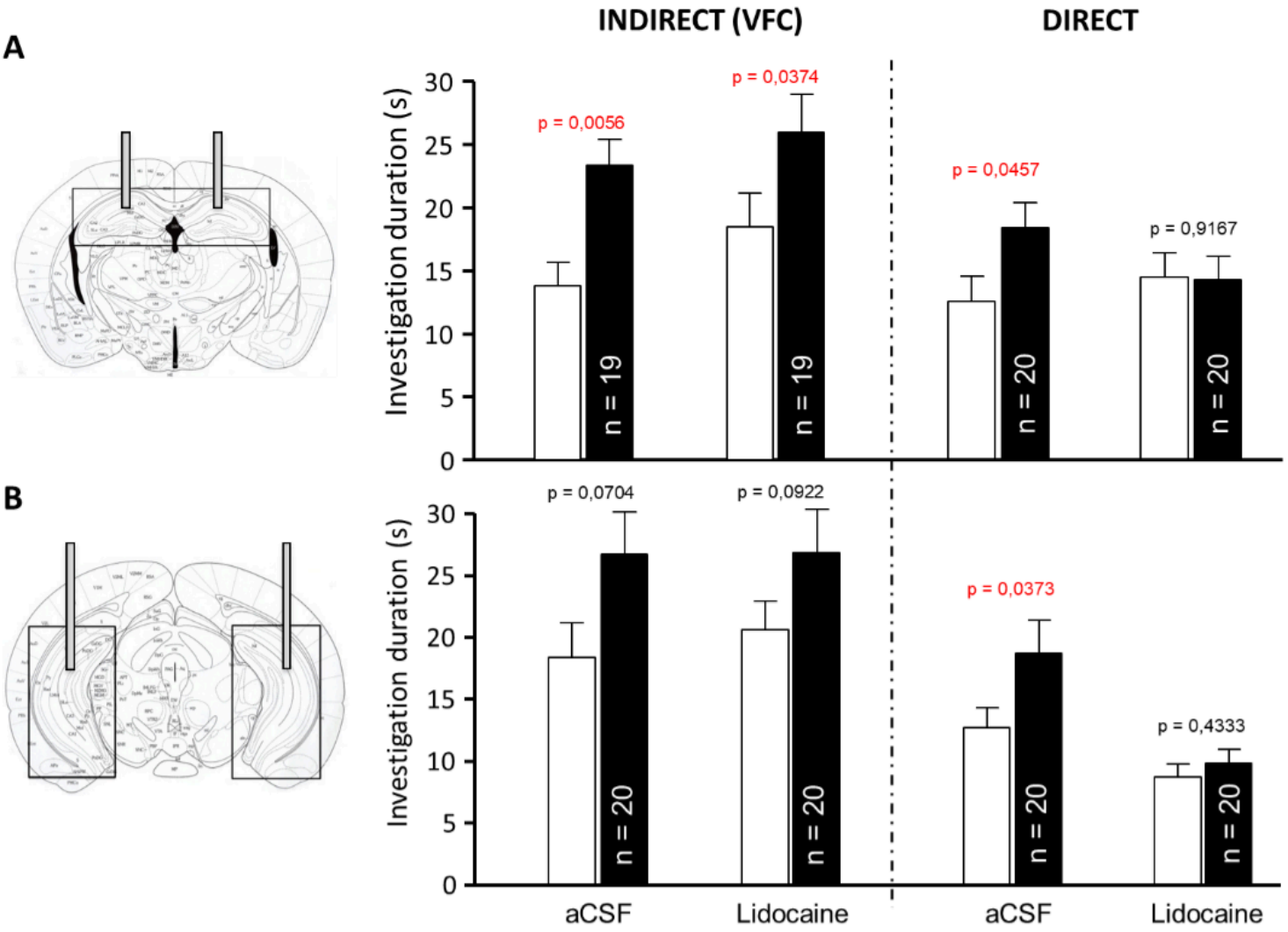


Figure 3

Behavioural performance measured during the choice sessions. **A** Results of animals infused in the dorsal hippocampus with either aCSF or lidocaine before the sampling session. Stimulus animal exposure was performed either indirectly, in the volatile fraction cage (VFC), or directly. White bars represent the investigation duration of/towards the familiar stimulus animal, black bars show investigation duration of/towards the non-familiar stimulus animal. The black bars provide also the number of animals per group. Mean + SEM is represented and p-values of the paired t-test statistical analysis are given. **B** Results of animals infused in the ventral hippocampus with either aCSF or lidocaine before the sampling session. Stimulus animal exposure was performed either indirectly, in the volatile fraction cage (VFC), or directly. Mean + SEM is represented and the p-values of the paired t-test statistical analysis are given.

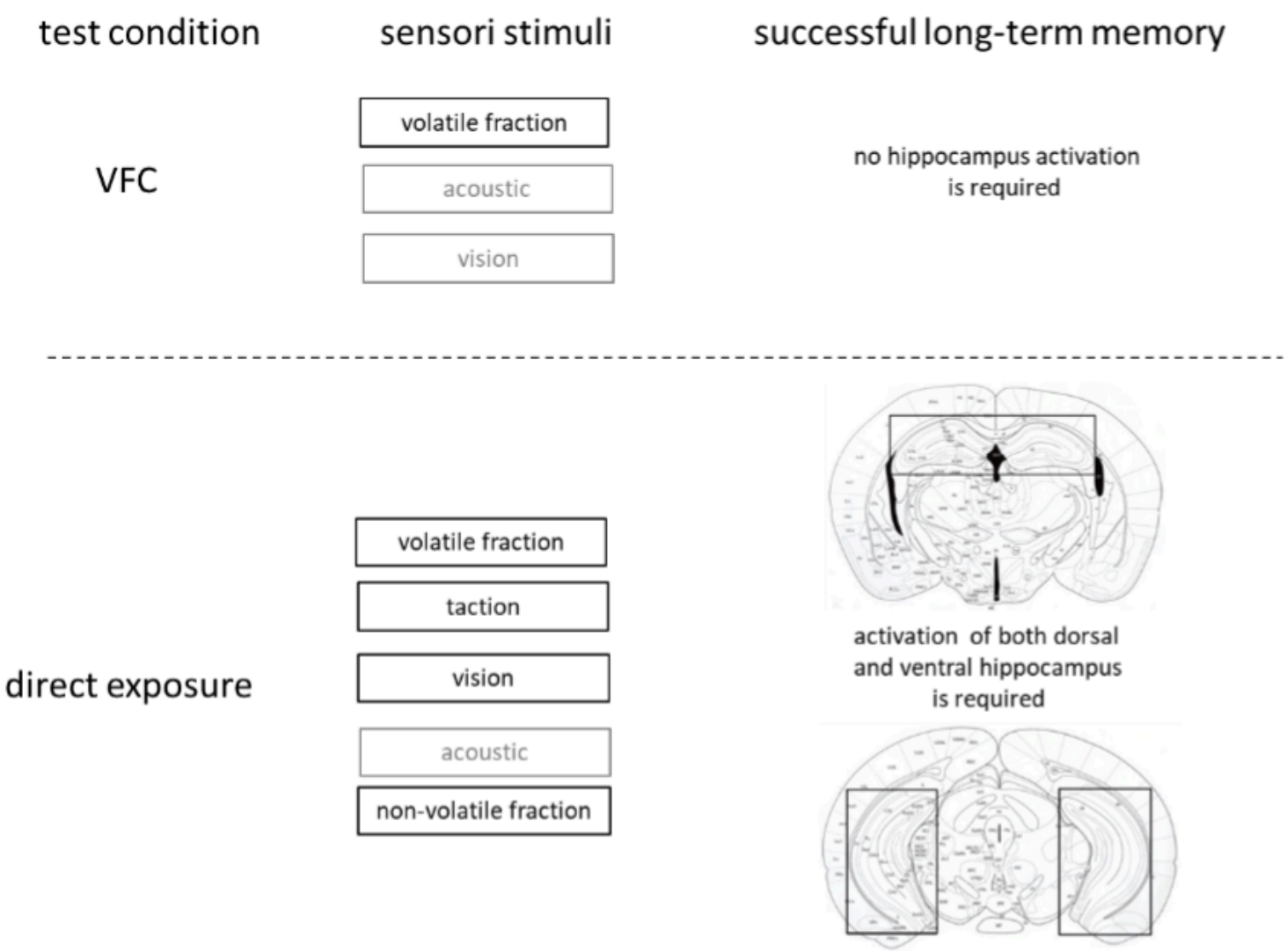


Figure 4

Schematic illustration summarising the results of the present study. It is suggested that in the VFC the long-term recognition is almost exclusively based on the volatile fraction of the olfactory signature and – thus – may not require the activation of the dorsal and/or ventral hippocampus. In contrast, direct stimulus animal exposure activates not only the olfactory sensory modality (and this time not only via volatile odours but also via non-volatile odours) but also the optic, tactile, and (to a limited amount) also the acoustic sensory modalities. Based on the results of the present study and previous findings, we hypothesise that both parts of the hippocampus are involved in the generation of a complex neuronal representation of the conspecific, which is then the basis of social recognition and should be called “social signature”.

Supplementary Files

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