Neuroscience Letters 511 (2012) 38–42

Contents lists available at SciVerse ScienceDirect

Neuroscience Letters

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

Hippocampal neurotransmitter efflux during one-trial novel object recognition in rats

Emily M. Stanley, Marlene A. Wilson, Jim R. Fadel*

Department of Pharmacology, Physiology and Neuroscience, University of South Carolina School of Medicine, Columbia, SC 29208, USA

ARTICLE INFO

Article history:
Received 15 August 2011
Received in revised form 13 January 2012
Accepted 14 January 2012

Keywords:
Hippocampus
Microdialysis
Acetylcholine
Glutamate
GABA
Memory

ABSTRACT

Several lines of evidence point to a role for the hippocampal formation and contiguous temporal lobe structures in a variety of learning and memory paradigms. Presumably, these cognitive phenomena are mediated (and accompanied) by dynamic changes in neurochemical transmission that may differ between learning and recall phases. However, the neurotransmitter correlates of most memory-related tasks have not been thoroughly investigated. Here we used a one-trial object recognition paradigm paired with in vivo microdialysis to assess hippocampal acetylcholine (ACh), glutamate and GABA efflux when rats were exposed to familiar objects, and when given the option to explore familiar and novel objects. Rats preferentially explored the novel object over the familiar one when presented with the option. Regardless of object familiarity, object exploration was accompanied by an increase in hippocampal ACh efflux, while GABA efflux was unaffected. However, glutamate efflux was not increased above baseline levels by presentation of familiar objects, but was significantly enhanced in the presence of the novel object. These data suggest that the hippocampus, and in particular, hippocampal glutamate, may be involved in memory processes during novelty recognition paradigms.

1. Introduction

The hippocampal formation mediates several aspects of cognitive function, including spatial learning, the formation of new memories and retrieval of stored memories [16]. Novel object recognition is a commonly employed test of memory that relies on rodents' inherent preference for exploration of new versus previously exposed stimuli [1] and requires very little training [11] other than habituation to the testing arena. The relative contribution of the hippocampal formation itself versus other adjacent and interconnected temporal lobe structures is not without controversy [6,10,17,25]. Clark et al. for example, showed that selective hippocampal lesions impair performance in a visual recognition task of spontaneous novelty preference when delays were longer than 1 min [8]. Moreover, studies have shown that when lesions encompass greater than 75–90% of the hippocampus, one-trial object recognition is significantly impaired [5], further supporting the stance that the hippocampus plays a crucial role in object recognition. Mumby and colleagues, however, found that hippocampal lesions disrupted novelty preference only in place or context trials, and not for the object itself [18], and others have suggested that impairments following total hippocampal lesions reflect deficits in object exploration during the sample phase (and hence, encoding), rather than object recognition memory [2]. Thus, the precise role of the hippocampus in object recognition memory remains to be elucidated but may be facilitated by a better understanding of phasic neurotransmitter release during different aspects of this memory task.

Dynamic, activity-dependent alterations in various neurotransmitters within the hippocampal formation are necessary facilitators of learning and memory processes. Glutamate, GABA and acetylcholine (ACh) play important roles in hippocampal memory formation, as is evidenced by the impairment of learning following antagonist administration for their respective receptors [4,7,10,19,23]. With regard specifically to novel object recognition, a number of studies (summarized in review by Dere et al., 2007) [10] suggest a role for hippocampal NMDA- and AMPA receptor-mediated transmission in object recognition at longer delays, indicating the importance of glutamatergic transmission within this behavioral paradigm.

While the spatial constraints of in vivo microdialysis limit the feasibility of combining this technique with certain behavioral paradigms, novel environment/object recognition tasks paired with simultaneous neurochemical assessment [9,13,14] have provided beneficial insights on the neurochemical correlates of exploratory and attentional activities linked to hippocampal

Abbreviations: ACh, acetylcholine; DNMS, delayed non-match to sample; glu, glutamate.

* Corresponding author at: Department of Pharmacology, Physiology and Neuroscience, University of South Carolina School of Medicine, Building 1, Room D10, Columbia, SC 29208, USA. Tel.: +1 803 216 3505; fax: +1 803 216 3538.
E-mail address: Jim.Fadel@uscmed.sc.edu (J.R. Fadel).

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doi:10.1016/j.neulet.2012.01.033

learning and memory processes. Many of the studies looking at behavior paired with microdialysis focus on the cholinergic system, but relatively little is known about the dynamic changes in hippocampal glutamate and GABA during object recognition. Here, we paired one-trial novel object recognition using an extended (90 min) delay, with in vivo microdialysis sampling in the hippocampus. The aim of this study was to determine the hippocampal neurochemical correlates of novel object recognition in a rodent model by simultaneous assessment of ACh, glutamate and GABA efflux in area CA1 of the ventral hippocampus.

2. Materials and methods

2.1. Animals

All animal care and use procedures were carried out in accordance with protocols written under the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee at the University of South Carolina. Nine young adult (3–5 months) male Fisher 344 Brown Norway F1 hybrid rats (National Institute of Aging breeding colony; Baltimore, MD, USA) were fed standard rat chow ad libitum and pair-housed on a 12:12 light–dark cycle (lights on at 0600 h) in a climate-controlled facility (temperature range 20–26 °C; relative humidity 30–70%). All experiments were conducted during the light cycle.

2.2. Novel object recognition during microdialysis

Under sodium pentobarbital anesthesia (60–70 mg/kg) all rats received unilateral implantation of guide cannulae (Bioanalytical Systems, Inc. (BAS); West Lafayette, IN, USA) in the dorsal hippocampus in the following coordinates relative to Bregma: –posterior −5.2 mm, lateral +3.8 mm at 10° angle, ventral −3.6 mm [20]. After a two day recovery period following stereotaxic surgery, rats were habituated to the microdialysis bowls for four consecutive days prior to the onset of microdialysis sampling. During habituation periods, each rat was exposed to two identical objects. These objects were adhered by Velcro approximately 180° from one another on the inside of the circular microdialysis bowl walls and approximately 2 inches from the base of the bowl. There was no barrier between the rats and the objects; thus, rats were able to approach and touch the objects during the entire presentation period. Rats were exposed to the same identical objects for one, 15 min period during each habituation day. Familiar objects were counterbalanced between subjects to account for any unforeseen inherent preference (Fig. 1), and consisted of a black rubber and plastic bottle stopper (2.5″ diameter, 1.5″ deep) or two intercrossed white nylon chew toys (4.5″ long, 1″ wide, 0.5″ deep). Following 4 days of habituation to the now familiar objects, rats underwent in vivo microdialysis in which 15 dialysates were collected in 15 min fractions. Following four baseline collections, rats were presented with the two familiar objects they had been habituated to on the previous four days. To insert the objects into the microdialysis bowl each rat was picked up while the objects were adhered (identically to the placement procedure during the 4 habituation days), and placed back in the microdialysis bowl equidistant from both objects with its head positioned 90° from both objects. At the cessation of the fifth collection, the objects were removed. Ninety minutes later (during collection 12), objects were placed back into the microdialysis bowls, but this time one familiar and one novel object were presented to each rat. Object recognition behaviors were recorded using a video camera for 15 min during collections 5 and 12. The amount of time each animal spent exploring each object was recorded (seconds), and the number of approaches to each object was later scored by two investigators from the video tapes and the results averaged. An active approach or investigation was defined as any exploratory behavior oriented toward, and occurring in close proximity (approximately two centimeters or less) to, either object.

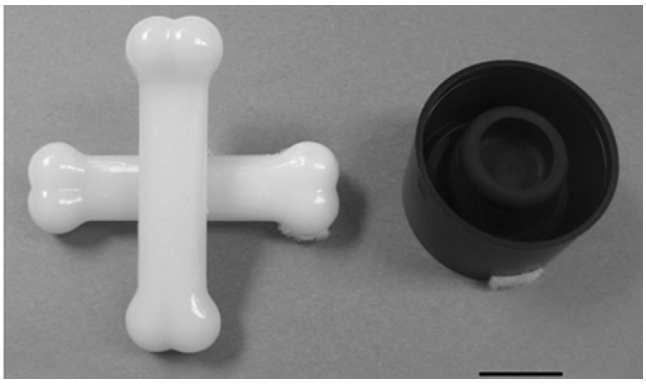

Fig. 1. Objects used for novel object recognition during in vivo microdialysis sampling consisted of intercrossed nylon chew bones (left) and rubber and plastic bottle stoppers (right); scale bar = 1 inch. The assignment of each object as novel or familiar was counterbalanced across subjects to control for the possibility of innate preferences.

At the conclusion of dialysis sessions animals were euthanized, and brains were removed. Probe placement was assessed on coronal sections through the hippocampus using an acetylcholinesterase background stain (see Supplemental Data). Animals with probe tracts outside of the target region were excluded from results. Microdialysis samples were stored at −80 °C until analysis by liquid chromatography with electrochemical detection as previously described for ACh [12] and amino acids [22]. Two animals were excluded from amino acid sampling due to uncontrollable bleeding at the probe site.

2.3. Statistics

All in vivo microdialysis data were expressed as a percentage of mean baseline values for each animal. Data were analyzed using a repeated measures ANOVA. Significant main effects were followed by paired samples *t*-tests between collection four (the final baseline) and all subsequent collections, and between collections five and 12. Behavioral data was analyzed using paired samples *t*-tests. Pearson coefficients were calculated to determine correlative relationships between neurotransmitter efflux and object exploration time. Significant main effects were defined by $P < 0.05$ and all statistical analyses were performed using SPSS for windows (V.17.0, SPSS Inc.; Chicago, IL).

3. Results

3.1. Exploratory behavior indicates a preference for novel object exploration

The analysis of time spent examining (Fig. 2A), and number of approaches to (Fig. 2B), novel and familiar objects during microdialysis sampling revealed a significant preference for the investigation of the novel object during collection 12. During exposure to two familiar objects in collection 5, rats spent 32.5 ± 5.1 s over 5.1 ± 0.1 separate approaches, exploring both objects. Following introduction of the novel object paired with the familiar object at collection 12 there was a significant preference for the exploration of the novel object in both approaches and time spent actively exploring the object ($t_8 = 5.487$ for time, $t_8 = 5.367$ for approaches; both $P = 0.001$). Rats spent 19.6 ± 4.1 s over 2.0 ± 0.1 approaches

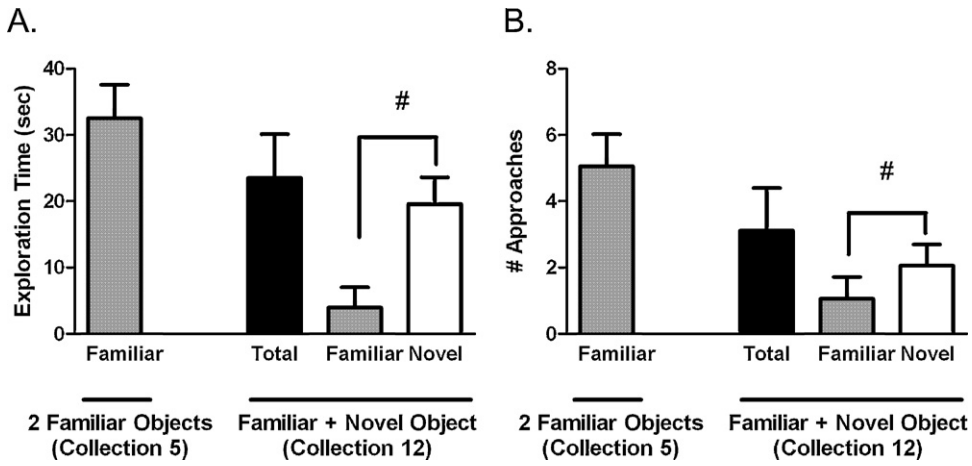

Fig. 2. Behavioral assessment of novel object recognition. (A) The total amount of time each animal spent exploring both objects was similar between collections 5 and 12. During presentation of both the novel and familiar objects during collection 12, rats spent significantly longer exploring the novel object. (B) Similar results were found in the number of approaches made towards objects, which indicated that rats preferentially approached novel objects more readily than familiar objects during collection 12. $^{\#}P \leq 0.001$; $n = 9$.

exploring the novel object while only exploring the familiar object for 3.9 ± 3.1 s over 1.0 ± 0.1 approaches. Importantly, the total time and approaches spent exploring the objects in collection 12 (23.5 ± 6.6 s over 3.1 ± 1.3 approaches) were not significantly different from total exploratory behavior in collection 5 when only familiar objects were present (P's for both time and approaches >0.3).

3.2. Novel object recognition differentially impacts hippocampal ACh, glutamate and GABA efflux

Hippocampal neurotransmitter efflux (Fig. 3) was assessed during presentation of familiar objects (collection 5), and subsequent introduction of one novel object paired with the familiar object (collection 12) 90 min later. Presentation of both the novel and familiar objects significantly increased CA1 ACh efflux (Fig. 3A) during the course of microdialysis sampling as indicated by a significant effect of TIME in the repeated measures ANOVA ($F_{14,126} = 5.950$; $P < 0.001$). Post hoc analysis indicated that ACh efflux was increased at the onset of familiar object presentation at collection 5, and remained elevated until collection 9. When the novel object was presented alongside the familiar object, a similar increase in ACh efflux was observed from collection 12 to 13, and again at collection 15. Furthermore, ACh efflux during the collection immediately following novel + familiar object presentation (collection 13) was significantly correlated with novel object exploration time ($r = 0.731$; $n = 9$; $P = 0.025$). The magnitude of ACh efflux was not different when comparing time point 5 (familiar object) to time point 12 (novel + familiar object).

Similar to ACh efflux, the repeated measures ANOVA for glutamate (Fig. 3B) indicated a significant effect of TIME during the course of dialysis sampling ($F_{14,84} = 3.744$; $P < 0.001$). Post hoc analysis revealed a gradual increase in glutamate efflux that reached statistical significance at collection 11, plus a significant enhancement in glutamate efflux following introduction of the novel object at collection 12, which persisted throughout the remainder of microdialysis sampling. Importantly, the magnitude of glutamate efflux was significantly greater following novel object introduction at collection 12 compared to familiar object association at collection 5. There were also strong trends for correlations between glutamate efflux during collections 12 and 13 (during and immediately after novel + familiar object presentation) and novel object exploration time (both r's > 0.61; both P's < 0.08).

Unlike ACh and glutamate efflux, hippocampal GABA efflux did not change during presentation of either familiar or novel objects (Fig. 3C).

4. Discussion

We have shown here that object exploration is associated with increased hippocampal ACh efflux regardless of the familiarity of the stimulus, whereas hippocampal glutamate is increased only in

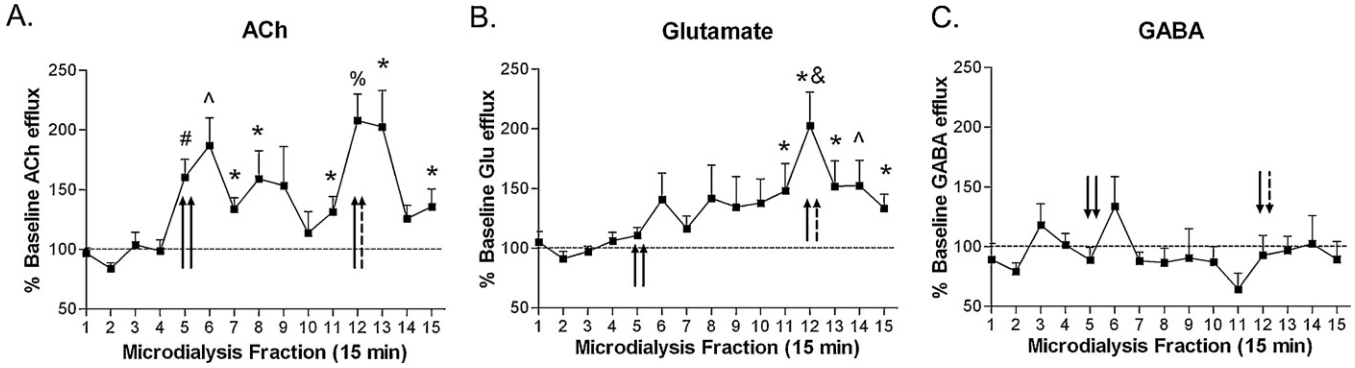

Fig. 3. Neurochemical analysis of novel object recognition behavior. (A) ACh efflux was significantly increased from baseline levels during both familiar and novel object presentation, while glutamate (glu) efflux (B) was increased compared to baseline and familiar object paradigms at the presentation of the novel object during collection 12. (C) GABA levels were not significantly altered during either familiar or novel object introduction. Solid arrows = familiar object presentation, dashed arrows = novel object presentation; $^{*}P \leq 0.05$, $^{\wedge}P \leq 0.005$, $^{\#}P \leq 0.001$, $^{\%}P \leq 0.0001$ compared to collection 4; $^{\&}P \leq 0.05$ compared to collection 5; $n = 9$ (ACh), $n = 7$ (amino acids).

response to novel object presentation. As anticipated, rats spent significantly longer investigating the novel object in comparison to the familiar when given the choice between the two. This preferential increase in glutamate efflux following exposure to novel, but not familiar objects, may indicate a role of for hippocampal glutamate in novelty signaling, particularly in a familiar context.

Previous literature has indicated that hippocampal ACh efflux is stimulated by exploration of a novel environment [13] and presentation of a novel object [9]. Our findings reinforce these reports, showing a similar degree of elevated ACh efflux following examination of both familiar and novel objects in a familiar context. The total amount of exploration time was similar during the initial (familiar) and subsequent (familiar plus novel) object presentations, suggesting that enhanced hippocampal ACh efflux in response to these stimuli reflects a cholinergic role in arousal and attentional aspects of object exploration [15,24]. However, only the immediate post-novel object period (collection 13) revealed a correlation between ACh efflux and novel object exploration time, suggesting that hippocampal ACh remains elevated in animals that had spent more time investigating the novel object during the previous 15 min period. This may reflect a cholinergic role in consolidational aspects of novel object memory formation [21]. Importantly, previous studies have shown cholinergic antagonists impair performance during one-trial object recognition tasks, while agonists produce a marked improvement (see [10] for review), indicating dynamic alterations in cholinergic efflux are in fact necessary for object recognition. Thus, increased ACh efflux likely does not signal novelty, itself, but may be a necessary component of novel object recognition when elevated in concert with other hippocampal neurotransmitters, such as glutamate (see below).

While ACh efflux appeared to correlate with general exploratory behavior of objects, glutamate efflux more closely paralleled presentation of the novel object versus the familiar object during the two object presentation trials. When given the choice between the two, all rats explored the novel object to a higher degree than the familiar object, and this increased preference for the novel object was accompanied by a significant increase in hippocampal glutamate efflux. Interestingly, this effect appears to be specific for novel object recognition, as exploration of a novel environment has been shown to produce no alterations in hippocampal or cortical glutamate neurotransmission [13], suggesting that glutamate may be involved in discriminative choice aspects (novel vs. familiar) of object exploration, rather than simply a response to any novel context or environment. Consistent with this hypothesis, glutamate efflux during collections 12 and 13 tended (statistical significance may have been achieved with more animals) to correlate with novel object exploration time. This finding also reinforces studies showing impaired performance in one-trial object recognition following the administration of AMPA and NMDA receptor antagonists (see [10] for review).

Like glutamate, hippocampal GABA release has been shown to be unaltered by exposure to a novel environment [13]. However, our results show that unlike ACh and glutamate efflux, GABA efflux was unchanged following presentation of familiar and novel objects during in vivo microdialysis sampling. This finding, however, does not imply that hippocampal GABAergic transmission is not crucial in the evaluation of familiar and novel objects. Rather, it may be that the spatiotemporal characteristics of microdialysis sampling are unable to detect rapid transient changes in GABA efflux or that different sources of GABA (e.g. septohippocampal inputs vs. local interneurons) are changing in opposite directions, resulting in no net effect.

Due to the nature of the experimental paradigm, rats were subjected to brief handling (<15 s) during the placement and removal of objects in the microdialysis bowls. While this simple act of handling may account for the elevations in ACh efflux (which was the only neurotransmitter evaluated to show significant increases in both object trials), Degroot and colleagues [9] used a similar protocol and found that increased ACh efflux was only observed in rats that actively manipulated the novel object. In our experiment all rats actively explored objects during both trials; therefore we speculate that the alterations in cholinergic transmission are more likely the result of object exploration than the brief handling episodes. Similarly, although no studies have looked at the effects of handling on hippocampal glutamate neurotransmission, a mild stressful (10 min) handling event produced no alteration in the prefrontal cortex, ventral tegmental area, or locus coeruleus during in vivo microdialysis sampling [26]. Moreover, the brief handling event required for object placement appeared to have no significant effect on glutamate efflux as no obvious effects were seen in the first exploration trial during collection 5. Therefore, it is unlikely that the brief handling event had any confounding effects on glutamate assessment during object recognition.

Previous experiments indicated that acute exposure to a novel object enhances performance on a radial arm maze task [9], which the authors attributed to increases in hippocampal ACh following object introduction. While that study used object recognition as an environmental enrichment factor, and our focus was on one-trial object recognition, we speculate that glutamate (possibly in concert with ACh) may be the key neuromodulator in the enhancement in cognitive performance, since the cholinergic system responds to any salient stimulus regardless of familiarity. Another recent study used a delayed non-match to sample (DMNS) object recognition task during microdialysis to assess hippocampal ACh [14], but to our knowledge, this is one of the first studies to assess hippocampal glutamate and GABA efflux during a one-trial novel object recognition paradigm. Unlike the DMNS task, the one-trial novel object recognition paradigm used here does not require spatial learning, stress-inducing food or water deprivation, or the acquisition and recall of response-reward associations. Furthermore, the relative simplicity of the one-trial schematic is proposed to be more tightly connected to normal human memory processes [11] and is a particularly quick and useful assessment of pharmacotherapies targeted at cognitive performance [10].

Our data provides the first reported finding where the hippocampal neurochemistry coincides with the accurate distinction in novel from familiar objects during behavioral assessment. The distinct nature of glutamate efflux in the recognition of novel objects suggests that the hippocampus does, in fact, play a role in object recognition. In particular, the implications of these data are reinforced by reports of increased hippocampal c-Fos expression following exposure to novel, but not familiar objects [3], and implies that glutamatergic efflux within the hippocampus plays an important role in one-trial object recognition memory.

In conclusion, we propose that one-trial novel object recognition screening in tandem with in vivo microdialysis sampling represents a feasible approach to delineating the neurochemical correlates of hippocampal-dependent cognition, and thus could be a useful tool in gaining a better understanding of the neurochemical basis of cognitive decline associated with various disorders and neurodegenerative states.

Conflicts of interest

The authors have no actual or potential conflicts of interest to disclose.

Acknowledgements

The authors thank Drs. Sandra J. Kelly and R. Charles Lawrence for valuable insights on the construction of the novel object

paradigm and for helpful comments on an earlier draft of the manuscript. The technical assistance of Hannah van Patten is gratefully acknowledged. This work was supported in part by National Institutes of Health grant R01AG030646 (JRF).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neulet.2012.01.033.

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