ENTWICKLUNG EINES OLFAKTORISCHEN GEDÄCHTNISTESTS FÜR LABORMÄUSE

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Dissertation an der Universität Hamburg, Deutschland, 2004

A NEW ONE TRIAL OLFACTORY RECOGNITION TEST FOR LABMICE

Dissertation at the department of Biology of the University of Hamburg, Germany, 2004

German with English abstract

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Verlag: **Litis Press Publishers**, München 2004
1. Auflage 11/2004
132 Seiten, 37 s/w Abbildungen, 36 Tabellen
Email: **info@litispress.com**

Der deutschen VLB Verzeichnis Lieferbarer Bücher gemeldet

Bibliografische Information Der Deutschen Bibliothek
Die Deutsche Bibliothek verzeichnet diese Publikation in der Deutschen Nationalbibliografie; detaillierte bibliografische Daten sind im Internet über http://dnb.ddb.de abrufbar.

Bibliographic information published by Die Deutsche Bibliothek
Die Deutsche Bibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data are available in the Internet at http://dnb.ddb.de.

Information bibliographique de Die Deutsche Bibliothek
Die Deutsche Bibliothek a répertorié cette publication dans la Deutsche Nationalbibliografie; les données bibliographiques détaillées peuvent être consultées sur Internet à l'adresse http://dnb.ddb.de.

ISBN 0-9751285-2-3

9 780975 128527

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Abstract

Many animal models in biomedical science are basing on laboratory mice. Cognitive sciences in recent years make increasing usage of methods that manipulate the genome of laboratory mice. For example studies on learning and memory have used knock-out mice, which had genes deleted that might be involved in memory processes.

Learning and memory tests need to take into consideration the natural behavioural predispositions of the animal species used. Thus I designed a memory test which takes into account the natural behaviour of mice.

(A) Test design

Laboratory C57 mice and Cannabinoid receptor 1-knock-out (CB1-ko) mice were used in this study. Stressful negative stimuli were strongly avoided. Because CB1-ko mice have a higher threshold to food deprivation than their wild type siblings (CB1-wt), food deprivation and food rewards were not used. The test focused on the natural tendency of many mammals to inspect thoroughly new objects in their environment. If the animal is confronted with the same object again, the time of inspection is significantly shorter. Obviously the animals had made themselves familiar with objects by investigative behaviour and they had stored them in memory as „known“ for some time.

Several points were taken into account in the test design:

- In mice the sense of smell plays a more important role than the sense of vision especially in social interactions, foraging activities and anti predator behaviour. Consequently, an olfactory memory test was developed.
- Since animals inspect new objects more intensely in a relaxed situation, the experimental set-up avoided stressful situations.
- Investigations were conducted into whether natural essential oils were more attractive to mice than monomolecular odours. Because essential oils were found not to be more attractive, monomolecular odours were used, which guaranteed a constant quality over time.
- Three odour pairs were chosen. Each consisted of two monomolecular odours of equal attractiveness: (-)-Carvon / Isoamyl Acetate, Allylcapronate / Linalool and Anethol / Amyl Propionate.
- Each trial with a given mouse involved a *learning period*, an *inter-test interval* (ITI) and the actual *test period*. The odours were presented in a 1.5ml reaction tube. In the *learning period* two identical odours were introduced to the mouse. In the *test period* the mouse was confronted with the odour from the preceding *learning period* in

one tube and an unknown odour in the complementary tube. In consecutive trials with the same mouse the ITI between *learning* and *test phase* was varied. Thereby a time course of memory performance could be covered.

- The memory performance was quoted as the index of the relative inspection duration (RID), which was calculated as the ratio of the inspection duration of the unfamiliar odour to the total inspection duration. If the median of the RID over all mice was significantly greater than 0.5, then the unfamiliar odour was inspected for longer, leading to the conclusion that the mice remembered the familiar odour. Each mouse was tested with each of the three odour pairs and each of the three ITI´s (systematically varied). In each of the ITI´s there were test naive mice, mice tested once and mice tested twice.
- As laboratory mice tend to be nocturnal, all tests were carried out in the dark phase under infrared light.

(B) Test applications

Two memory tests were carried out, each with 18 male C57 mice, and two further tests each with 9 male CB1-ko and 9 CB1-wt controls.

The results of the memory tests with C57 and CB1-wt mice showed that the test was well suited to investigate (1) if and for how long olfactory stimuli could be recalled and (2) whether memory performance depended on earlier experiences.

CB1-ko mice are known to show a higher and longer freezing reaction to conditioned aversive stimuli than their CB1-wt siblings. Hence, extended memory performance was expected.

However, no recognition of the familiar odour could be found in the CB1-ko mice after any ITI, whereas CB1-wt mice recognized the familiar odour after 1 h and 72 h.

The inspection duration of the CB1-ko mice was significantly shorter at any time compared to their wt siblings. Furthermore ko mice had a significant higher level of defecation. Both results lead to the conclusion that ko mice were more anxious than their wt controls. Ko and wt mice were in a different physiological state. Hence, the test results for the CB1-ko mice could not be interpreted clearly.

Tests with C57 and CB1-wt mice showed that the familiar odour was remembered after 1h, 3h and 72h.

The analysis of the 24 h ITI tests revealed a complex picture. Mice which were tested naïve at the start of the 24 h memory test remembered the familiar odour in the test phase, however test-experienced mice did not.

The lack of olfactory memory found for experienced mice after 24 h can be interpreted as **phasic memory**. This "Kamin effect" has also been discovered in other animal species and is commonly regarded as an expression of memory consolidation.

On the contrary to experienced mice, naïve mice memorised the previously presented odour after 24 hours. This unexpected result is discussed as suggesting that the consolidation of odour memory is dependent on the significance of the stimulus. For naïve mice their first odour encounter was impressive enough for the memory to be consolidated and recallable after 24 h. For experienced mice on the other hand, the learning phase in the 24 h test has been at least their second encounter with this test situation. It was not as impressive and therefore, the memory was not yet consolidated and recallable after 24 h. This interpretation assumes a value dependency of the time course of memory consolidation: The more significant an encounter (incident) is, the earlier the consolidation takes place and therefore the earlier the memory is recallable.