Reducing forebrain calcineurin activity facilitates reversal learning in the Y maze

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Abstract

The protein phosphatase calcineurin (CaN) has been recognized as a molecular constraint on memory formation. Here we test whether this is true for learning and reversal learning in a Y-maze reference task. We used a transgenic approach to inhibit CaN activity in forebrain neurons via the induced expression of a CaN inhibitor. Training mice in a spatial Y-maze test with reduced CaN activity did not affect rate of acquisition. In contrast, rate of acquisition was significantly facilitated when CaN activity was reduced during reversal training. These data show that, in case of Y-maze learning, the impact of reduced CaN activity is reversal learning specific.
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Main text

The hippocampus is one of the key structures in the brain that encodes both spatial and non-spatial information. It plays a critical role in encoding sensory input and controlling the output leading to correct behavioural responses (for recent reviews see Rolls and Kesner, 2006; Martin and Clark, 2007). Besides encoding novel information, the hippocampus is also involved in detecting changes in a familiar environment or situation (so called novelty detection) and the adaptation of the behavioral output to cope with the changes in a familiar environment (for review see Lisman and Grace, 2005). It is suggested that the detection of changes and the adaptation to changed elements in a familiar environment require multiple processes in contrast to the acquisition of the original discrimination (McAlonan and Brown, 2003). It requires inhibition of the previously learned response, as well as the acquisition of a response adapted to a new set of contingencies (Watson et al., 2006). Because these processes are more complex than the original learning, it is suggested that the detection and adaptation to changes in a familiar environment require more from the hippocampus. In line with this, lesion studies have shown that reversal learning (e.g. the relocation of a food reward) is more sensitive to hippocampal dysfunctioning or damage compared to the original learning (Murray and Ridley, 1999). In addition, Bannerman and colleagues (2003) showed that mice lacking the GluR1 subunit of the AMPA receptor were unimpaired during acquisition of a spatial discrimination task, but displayed a mild but significant deficit during reversal of the spatial discrimination. Similarly in a Y-maze spatial discrimination test, two year old mice show deficits in reversal learning, but not during the original learning phase of the task (Havekes and Van der Zee, unpublished observations).

Changes in synaptic efficacy are known to be important for the processing of (novel) information and memory storage. These changes are controlled by the phosphorylation state of intracellular proteins and are bidirectionally regulated, partly by the opposing action of protein kinases and protein phosphatases (reviewed in Lee, 2006). While it is generally acknowledged that protein phosphorylation by protein kinases is critical for memory encoding, the role of protein phosphatases remains a matter of debate. One hypothesis is that the calcium sensitive protein phosphatase calcineurin (CaN, also known as protein phosphatase 2B) acts as a molecular constraint on memory formation, based on the learning performance of transgenic mice with altered CaN activity (Mansuy et al., 1998a; Mansuy et al., 1998b; Malleret et al., 2001). Alternatively, it is proposed that CaN is critically involved in situations where adaptation of a previously established behavioral response pattern is needed (Zeng et al., 2001, Lin et al., 2003; Havekes et al., 2006).

In this paper, we investigate the role of forebrain CaN in spatial learning and reversal learning in the Y maze using transgenic mice in which we could temporally restrict CaN activity via induced expression of a CaN inhibitor in the forebrain during training or reversal training.

Double transgenic male mice carrying the tetO promoter-AI transgene and CaMKIIα promoter-rtTA2 transgene were maintained on a C57Bl/6j background. Genotype verification was determined by PCR as described previously (Malleret et al., 2001;
Doxycycline administration (Dox, West-ward pharmaceutical, Eatontown, NJ; 6mg/g food) was used to induce the expression of the autoinhibitory domain in the C-terminus of the catalytic subunit of CaN (Malleret et al., 2001). RT-PCRs were performed as described previously using oligonucleotides specific for tetO-AI (Malleret et al., 2001). In accordance with previous findings by Malleret and colleagues (2001), one week of Dox treatment was sufficient to induce the expression of the CaN inhibitor transgene, while it was absent in the hippocampus from control mice that did not receive Dox (Fig.1A).

**Figure 1.** Reducing forebrain CaN activity has differential effects on learning and reversal learning. (A) Double transgenic mice fed with Dox for seven days express the CaN inhibitor. (B) Experimental protocol for training and reversal training in the Y maze. Mice of the TDox group received Dox during training. Mice of the T,RTDox group received Dox selectively during the reversal training. (C) Percentage of correct trials per session during 6 days of Y-maze training. (D) Percentage of correct trials per session during 8 days of reversal training. Error bars indicate ± SEM.
For the Y-maze experiment, 14 to 20 weeks old mice carrying both transgenes were used. They were individually housed in standard macrolon cages and maintained on a 12 hour light/ 12 hour dark cycle (lights on at 6.00 a.m.) with food (hopefarm® standard rodent pellets) and water available ad libitum. A layer of sawdust served as bedding. Subjects were food deprived to 90 % of their individual body weight under ad libitum feeding conditions, starting seven days before the beginning of the experiment. Mice were divided into three groups: a group that did not receive Dox (control group; n = 11), a group that received Dox during training (TDox group; n=13), and a group that received Dox during reversal training (T,RTDox group, n=11). The scheme of Dox treatment for the different groups is shown in figure 1B.

Habituation and training procedures in the Y maze were conducted as previously described (Havekes et al., 2006; Havekes et al., 2007; Van der Borght et al., 2007). In short, subjects were habituated to the Y maze. After habituation, mice received daily sessions of training consisting of six trials. During the entire training, either the right or left arm was baited (randomized, but consistently for an individual) and mice were allowed to visit one of the two accessible arms. A visit to the baited arm was recorded as a correct trial. Six days of training was followed by one week without testing. After this non-testing week, the control and T,RTDox group were subjected to eight days of reversal training, with the food reward located in the previously unrewarded arm.

The procedures described in the present study were approved by the Dutch Animal Experiment Committee of the University of Groningen in compliance with Dutch law and internal regulations. Statistical analyses used two factor ANOVAs with genotype as between-subject factor and session as within-subject factor. $P < 0.05$ was considered as significant. Data are expressed as mean ± S.E.M.

During Y-maze training, all groups progressively learned to locate the baited arm ($F_{5,165} = 24.489$, $P < 0.001$, Fig. 1C), resulting in a final score of 92.36 ± 3.76 % for the control group, 92.94 ± 3.25 % for the TDox group and 90.15 ± 3.70 % for the T,RTDox group. No group or interaction effects was found (group and interaction $F < 1$ in both cases) indicating that CaN inhibition did not affect the performance during training in the Y maze.

Decreasing CaN activity is expected to result in a shift in the balance of activity between protein kinases and protein phosphatases towards phosphorylation of substrates including those that are involved in memory formation. Yet, we did not find an increase in rate of performance during Y-maze training. We previously showed that hippocampal CaN activity and protein levels were decreased after Y-maze training (Havekes et al., 2006). Therefore, it is likely that this endogenously induced reduction of CaN activity during training also occurred in the Dox-treated mice. Apparently, further reduction of CaN activity via the expression of the CaN inhibitor does not facilitate memory formation for the location of the food reward during Y-maze training. During the first reversal training session, performance dropped to 6.06 ± 4.65 % and 9.09 ± 4.69 % for the control group and T,RTDox group respectively, demonstrating that mice of all groups still had a preference for the previously rewarded arm. With ongoing training, both groups shifted their preference to the newly rewarded arm ($F_{7,140} = 77.656$, $P < 0.001$, Fig.1D). Strikingly, in contrast to training there was a strong effect of CaN inhibition during reversal training in the T,RTDox group only.
(F(1,20) = 12.626, P< 0.005, Fig.1D). While the T,RTDox group reached an average performance of 90.90 ± 3.46 % after 8 sessions of reversal training, the control group reached an average performance of respectively 74.24 ± 3.45 %.

These findings imply that forebrain CaN acts as a molecular constraint in case of reversal learning in the Y maze. CaN inhibition in the forebrain has previously been shown to be beneficial for reversal learning in the spatial version of the Morris water maze (Malleret et al., 2001). Since PKA and CaN are thought of functioning antagonistically, reducing CaN activity could facilitate PKA-dependent processes, including the phosphorylation of key sites on AMPA receptors resulting in enhanced insertion and open channel time of GluR1-containing AMPA receptors (Banke et al., 2000). Reduced CaN activity levels can in addition diminish the activity of protein phosphatase 1 (Mulkey et al., 1994) and as a consequence enhance transcriptional activity by increased activation of the cyclic AMP responsive element binding protein (CREB) (Hagiwara et al., 1992).

We previously showed that reversal training was accompanied by enhanced PKA expression in hippocampal subregions (Havekes et al., 2007). These changes in PKA immunoreactivity were paralleled by enhanced levels of AMPA receptor phosphorylation at the GluR1 serine 845 site (Havekes et al., 2007), a site that is targeted by both PKA and CaN. Enhanced phosphorylation of the serine 845 is important for the integration and mean open probability of GluR1 containing AMPA receptors subunits (Banke et al., 2000; Esteban et al., 2003). Thus, it could be that by reducing CaN activity, PKA-induced phosphorylation of substrates like the S845 Glur1 site is facilitated, which favors the rate of acquisition during reversal training.

Here, we show that reducing forebrain CaN activity enhances behavioral performance in a Y maze specifically during reversal training. These data suggest that CaN indeed acts as a constraint on memory formation in case of reversal learning in the Y maze. Future research is needed to investigate in which of the multiple signal transduction cascades required for reversal learning CaN plays a crucial role.

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Author contributions

R.H. and E.A.V.d.Z. generated the hypotheses and designed the experiments. R.H., A.K.D.V and I.M.N. performed the experiments. R.H. wrote the manuscript with input from with input from E.A.V.d.Z., I.M.N. and P.G.M.L.
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