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Revisiting metaplasticity: The roles of calcineurin and histone deacetylation in unlearning odor preference memory in rat pups

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ABSTRACT

Previous work has shown that 24 h duration odor preference learning, induced by one-trial training, generates a down-regulation of the GluN1 receptor in anterior piriform cortex at 3 h, and results in metaplastic unlearning if a second training trial is given at 3 h. The GluN1 receptor upregulates at 24 h so 24 h spaced training is highly effective in extending memory duration. The present study replicates the piriform cortex unlearning result in the olfactory bulb circuit and further studies the relationship between the initial training strength and its associated metaplastic effect. Intrabulbar infusions that block calcineurin or inhibit histone deacetylation normally produce extended days-long memory. If given during training, they are not associated with GluN1 downregulation at 3 h and do not recruit an unlearning process at that time. The two memory strengthening protocols do not appear to interact, but are also not synergistic. These outcomes argue that it is critical to understand the metaplastic effects of training in order to optimize training protocols in the service of either memory strengthening or of memory weakening.

1. Introduction

Real life learning is not an isolated, stand-alone experience. Each learning event builds on previous experience and hence is unique in each individual. If learning is an accumulative, built-up process, then our memories cannot be accounted for by a single synaptic plasticity event during one experience. Metaplasticity describes the phenomenon by which the capacity for synaptic plasticity is altered by prior synaptic activity (Abraham and Bear, 1996). Thus metaplasticity is likely critically involved in complex learning and directly influences behavioral outcomes. However, how metaplasticity occurs *in vivo* in a way that is relevant to cognitive function is not well understood.

Previously we have demonstrated that metaplasticity occurs in a natural learning model – early odor preference learning in rat pups (Mukherjee, Harley, and Yuan, 2017; Mukherjee et al., 2014). Week-old rat pups form a preference to an odor that is paired with a tactile stimulus signaling maternal care (e.g. stroking using a brush) (Sullivan and Leon, 1986; Yuan, Shakhawat, and Harley, 2014). One trial, 10-min training can lead to a preference memory for the conditioned odor lasting up to 24 h. Increasing the training strength by multi-trial, 24 h spaced training extends the memory to days (Fontaine, Harley, and Yuan, 2013). However, two trials of training separated by 3 h actually prevent the odor preference memory (Mukherjee et al., 2014).

Interestingly, synaptic NMDA receptors (NMDARs) in the anterior piriform cortex (aPC) decrease at 3 h, while they increase at 24 h following one trial training (Mukherjee et al., 2014). The altered plasticity at 3 h post the initial training is likely induced by occurring at the time of NMDAR down-regulation. Reduced Ca^{2+} entry *via* decreased numbers of NMDARs during the 2nd associative training at 3 h results in depotentiation (or LTD) of the aPC synapses and unlearning of the previous experience (Mukherjee et al., 2014). Indeed, blocking NMDARs during the 2nd training prevents the unlearning (Mukherjee et al., 2014). Further work shows that NMDAR down-regulation is mediated by both mGluR5 and calcineurin signaling (Mukherjee et al., 2017). Blocking either aPC mGluR5 or calcineurin during the initial training also prevents the unlearning induced by the 2nd training, 3 h later (Mukherjee et al., 2017). The timing between the prior and subsequent training appears to be critical, as re-training at 24 h, when NMDA receptors are increased (Mukherjee et al., 2014), enhances memory (Fontaine et al., 2013).

Here we study the relationship between the strength of the initial learning and its metaplastic effect on subsequent training. We explore

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whether a stronger induction that produces prolonged memory has the same metaplastic effect on 3 h re-training. Two protocols have been established previously that induce prolonged odor preference memories. Blocking calcineurin with FK-506 (Christie-Fougere, Darby-King, Harley, and McLean, 2009) or blocking histone deacetylation with class I/II histone deacetylase (HDAC) inhibitor trichostatin A (TSA) (Bhattacharya et al., 2017) in the olfactory bulb (OB) extends one-trial odor preference memory for days. We have established that both the OB and aPC are plastic sites that are critical for early odor preference learning. NMDAR blockade in either site prevents odor preference memory formation (Lethbridge, Hou, Harley, and Yuan, 2012; Morrison, Fontaine, Harley, and Yuan, 2013). OB NMDARs are also down-regulated at 3 h following training (Lethbridge et al., 2012). Since both calcineurin (Mukherjee et al., 2017; Snyder et al., 2005) and histone deacetylation (Jayanthi et al., 2014) down-regulate NMDAR GluN1 subunit in other brain structures, here we first examine the effects of FK-506 or TSA OB infusion during single trial training on GluN1 expression and subsequent learning at 3 h. After establishing their effects on NMDAR regulation and unlearning, we examined possible cross-talk between calcineurin and histone acetylation in the OB.

2. Material and methods

2.1. Animals

Sprague Dawley (Charles River, Canada) rat pups of both sexes were used in this study. The day of birth was considered postnatal day (PND) 0. Litters were culled to 12 rat pups on PND1. Animals were kept in temperature-controlled rooms (20–25 °C) on reverse 12 h light/dark cycles. All experimental procedures were approved by the Institutional Animal Care Committee at Memorial University of Newfoundland following the guidelines set by the Canadian Council on Animal Care.

2.2. Experimental design

2.2.1. Cannula implantation and olfactory bulb infusion

On PND 5, rat pups were anaesthetized by hypothermia and placed in a stereotaxic apparatus. The skull was exposed and two small holes were drilled over the central region of each OB. The cannulae were implanted into the OB and cemented to the skull. The skin was sutured together and pups were allowed to recover on warm bedding before being returned to the dam. All drugs were infused into the OB on PND 6 at 20 min before the first training. 1.0 µl of a drug or vehicle was injected bilaterally into the OB for behavioral experiments using a 10 µl micro-syringe. In pups for quantitative immunoblotting, drugs were infused either bilaterally into the OB (Fig. 4) or in one side into the OB with vehicle infused into the contralateral bulb (Figs. 1–3, and 5–7).

2.2.2. Drug preparation

Pharmacological agents used include TSA (working concentration 0.05 µg/µl/OB as described earlier (Bhattacharya et al., 2017, dissolved in 10% DMSO; Cedarlane, Canada; Cat. No. T-1052), a calcineurin (phosphatase 2B) inhibitor FK-506 (5 mM, dissolved in 10% DMSO; Tocris; Cat. No. 3631) and a phosphatase 1/2A inhibitor okadaic acid (500 µM, dissolved in 10% DMSO; Calbiochem; Cat. No. 459620). The working concentrations for FK-506 and okadaic acid used produced the published results in our previous work (Mukherjee et al., 2017).

2.2.3. Odor preference training and testing procedure

2.2.3.1. Training. A single 10 min training session was performed on PND 6 rat pups in temperature controlled (28 °C) behaviour rooms. After the drug infusion, pups were placed on peppermint-scented bedding for 10 min and stroked with a paint brush for 30 s every other 30 s. Pups in the non-learning condition were placed on the peppermint-scented bedding for 10 min without stroking. Peppermint-scented bedding was prepared by adding 0.3 ml of peppermint extract (G.E. Barbour Inc., Canada) to 500 ml of regular unscented woodchip bedding. Pups were returned to the dam immediately after training until re-training, testing or sacrifice. For the re-training behaviour experiment, pups were re-trained at 3 h after the first training. Pups were re-exposed to peppermint-scented bedding while being stroked using the same procedure as in the first training. Pups were returned to the dam after re-training.

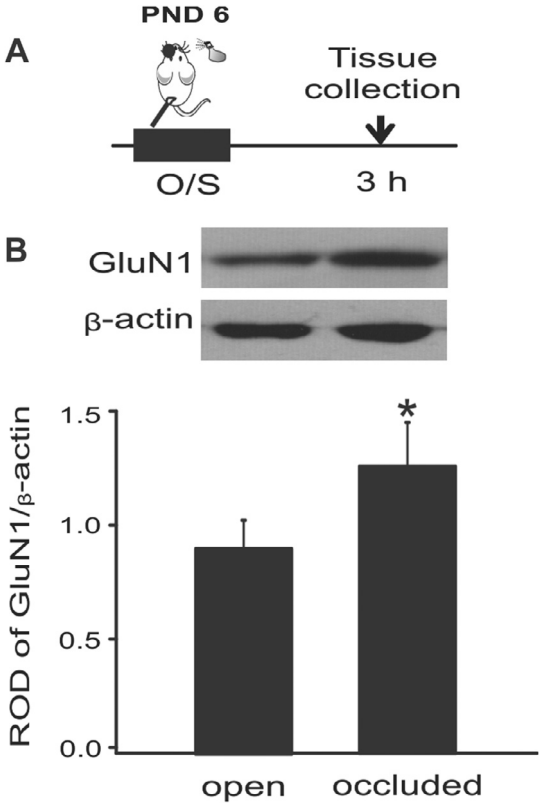

Fig. 1. Early odor preference learning down-regulates GluN1 expression in the OB. A. Schematic of training and tissue collection. O/S: Odor + Stroke training. B. GluN1 expression is reduced in the OB of open naris 3 h following odor preference learning. $^{*}p < 0.05$.

For the Fig. 1 experiment, unilateral naris occlusion was performed before the odor + stroking training as described previously (Fontaine et al., 2013; Mukherjee et al., 2017; Mukherjee et al., 2014). Nose plugs were constructed using polyethylene 20 tubing and silk surgical thread. 2% Xylocaine gel (AstraZeneca) was applied to the left naris of the pup. After a 3–5 min rest, the plug was gently inserted in the left naris of the pup. After 10-min habituation, the pup underwent training. The nose plug was removed immediately following training and the pup returned to dams.

2.2.3.2. Testing. Twenty-four hours following the initial training, pups were tested for odor preference memory by using a two-choice odor preference test. Testing was carried out in a stainless steel test box placed over two training boxes. For all of the tests, one training box contained peppermint-scented bedding, and the other contained normal, unscented bedding. Training boxes were separated by a 2 cm neutral zone. During testing, each rat pup, one at a time, was separated from the dam and transferred to a no bedding holding cage in the testing room to prevent odor contamination. To start the testing, the pup was placed in the neutral zone of the test box. The percentage of time spent over peppermint-scented bedding or normal bedding for each pup was recorded during each of five 1 min trials. Pups were given 30 s of resting time in a clean holding cage between each of the five 1 min trials.