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Temporal course of gene expression during motor memory formation in primary motor cortex of rats

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ABSTRACT

Motor learning is associated with plastic reorganization of neural networks in primary motor cortex (M1) that depends on changes in gene expression. Here, we investigate the temporal profile of these changes during motor memory formation in response to a skilled reaching task in rats. mRNA-levels were measured 1 h, 7 h and 24 h after the end of a training session using microarray technique. To assure learning specificity, trained animals were compared to a control group. In response to motor learning, genes are sequentially regulated with high time-point specificity and a shift from initial suppression to later activation. The majority of regulated genes can be linked to learning-related plasticity. In the gene-expression cascade following motor learning, three different steps can be defined: (1) an initial suppression of genes influencing gene transcription. (2) Expression of genes that support translation of mRNA in defined compartments. (3) Expression of genes that immediately mediates plastic changes. Gene expression peaks after 24 h – this is a much slower time-course when compared to hippocampus-dependent learning, where peaks of gene-expression can be observed 6–12 h after training ended.

1. Introduction

The primary motor cortex (M1) is thought to be one brain area where motor memories are formed and encoded (Monfils, Plautz, & Kleim, 2005). In response to motor training in rats, profound changes within the matrix of M1 have been described at multiple sites (Hosp, Pekanovic, Rioult-Pedotti, & Luft, 2011): at the cellular level, an increment in dendritic length and arborisation occurs in apical (Greenough, Larson, & Withers, 1985) and basal dendrites (Kolb, Cioe, & Comeau, 2008) of layer II/III and V motor neurons (Greenough et al., 1985; Withers & Greenough, 1989) contralateral to the trained limb. Furthermore, an initial increase in spine formation is followed by an enhanced turnover that reduces the number of spines to baseline levels but selectively preserves functionally relevant synapses (Xu et al., 2009). At the level of synaptic weights, motor skill learning induces a long-lasting increase of synaptic strength in M1 horizontal connections of layer II/III suggesting an association with long-term potentiation (LTP)-like plasticity (Rioult, 1998). In line with this assumption, capacity to induce LTP was reduced whereas long-term depression (LTD) was increased, suggesting that the learning-induced gain in synaptic strength reduced the capacity of LTP-formation (Rioult-Pedotti, Friedman, & Donoghue, 2000). Several weeks after skill acquisition, the ability to form LTP was restored while the horizontal connections of layer II/III remained strengthened (Rioult-Pedotti, Donoghue, & Dunaevsky, 2007). At the level of cortical physiology, motor learning induces an enlargement of the motor-cortical representation (motor maps) of the body-parts that became trained. This phenomenon can be observed in rodents, primates, and humans (Kleim, Barbay, & Nudo, 1998; Nudo, Milliken, Jenkins, & Merzenich, 1996; Pascual-Leone et al., 1995). This enlargement is learning specific as it does not occur in response to mere motor activation and its magnitude is proportional to learning success (Kleim et al., 2004; Molina-Luna, Hertler, Buitrago, & Luft, 2008).

De novo synthesis of proteins is required for most of plastic changes that occur during motor learning (Alvarez, Giuditta, & Koenig, 2000; Bisby & Tetzlaff, 1992) and a learning-specific hippocampal protein expression has been demonstrated in response to spatial learning in rats (Monopoli et al., 2011). In line with these

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findings, protein-synthesis inhibition in M1 interferes with the acquisition of a motor task in rats (Luft, Buitrago, Ringer, Dichgans, & Schulz, 2004).

Changes in gene expression are expected to precede the synthesis of novel proteins that further form the molecular basis of motor cortical neuroplasticity. Such changes have been demonstrated in the hippocampus of rats that were trained in the Morris water maze task (Cavallaro, D'Agata, Manickam, Dufour, & Alkon, 2002) and in a passive avoidance learning paradigm (D'Agata & Cavallaro, 2003). Regulated genes could be classified into the categories of "cell signalling", "synaptic proteins", "cytoskeletal proteins", "apoptosis" and "transcription and translation". Thus, these sets of regulated genes were ideally suited to mediate neuroplasticity processes including changes in morphology and synaptic weights (Monfils et al., 2005).

Besides the functional role of regulated genes, the temporal succession of gene regulating processes has to be taken into account, as gene-expression in memory formation is progressing through different stages (Alberini & Kandel, 2015; Paratore et al., 2006). For example, cascade-like alteration in gene expression has been observed within the hippocampus following passive avoidance learning (O'Sullivan et al., 2007). In the Morris water maze task, regulated genes within the hippocampus of animals belonging to the spatial learning group were largely overlapping with swimming controls but groups could be clearly distinguished due to the unique temporal profile of up- or down-regulation (Cavallaro, D'Agata, Manickam, Dufour, & Alkon, 2002). Thus, learning-specific gene expression is not only defined by the identity of regulated genes - but also by the temporal profile of their expression.

Recently, motor learning-related alterations in gene expression could also be demonstrated within M1 of rats that were trained in a reach and grasp task (Cheung et al., 2013). As Cheung and colleagues focused on a single time-point during memory stabilization, the unique temporal profile and identity of regulated genes during early skill acquisition is still unknown.

As we hypothesized that gene regulation also occurs in non-discrete fashion early after motor skill acquisition, the objective of this study was to determine this temporal profile of changes in gene-expression within M1 in response to motor skill learning. We therefore assessed motor cortical mRNA levels of rats that were trained in a skilled reaching task using a microarray 1 h, 7 h and 24 h after the end of the second training session – the time-point where the steepest phase of learning occurs (Buitrago, Ringer, Schulz, Dichgans, & Luft, 2004). To assure learning specificity of changes, mRNA levels of trained animals were related to a control group.

2. Materials and methods

2.1. Animals and experiments

Twenty-one adult male Long–Evans rats (8–12 weeks old, raised within our own stock) were used in this study. The Animal Care and Use Committee of the State of Baden-Württemberg (Germany) approved all animal procedures. The rats were randomly assigned to groups trained either in a skilled reaching task (SRT) or a control task (CT) for 2 days. Trainings were performed at the beginning of the dark phase of a 12 h day/night cycle. For both tasks, exposure to a customized training cage, food, handling and pre-training were identical. Animals were euthanized 1 h (n = 4 for SRT and n = 3 for CT), 7 h (n = 4 per group) or 24 h (n = 3 per group) after training session two. The brains were removed for tissue processing.

2.2. Experimental setup and behavioural experiments

Training sessions were performed at the beginning of the dark phase. Animals were food-restricted for 24 h before the first pre-training session. During training animals were kept slightly over their initial weight (336.7 ± 31.2 g) by providing 50 mg/kg of standard lab diet after each training session. Water was given ad libitum. The reaching task was performed as previously described (Buitrago et al., 2004). The training cage was a 15 × 40 cm chamber (height 30 cm) with a vertical window (1 cm wide, 5 cm high, lower edge 2 cm above ground) in the front wall and a small light sensor in the rear wall (7 cm above ground). Animals were first pre-trained for five days learning to open the motorized sliding door that covered the front window by nose-poking the sensor in the rear. Opening the window gave access to one food pellet (45 mg, Bio-serve, Frenchtown, NJ, USA) located on a small horizontal board in a distance of 0.5 cm relative to the outside edge of the window. During pre-training pellets were retrieved by tongue. Upon retrieval a pellet dispenser automatically replaced the pellet. In SRT rats, pre-training was followed by motor skill training that was initiated by removing the board and placing the pellet on a small vertical pedestal 1.5 cm away from the window. In this position pellets were only retrievable by using the forelimb. Because the diameter of the pedestal was approximately that of the pellet, the pellet was in an unstable position and easily kicked off. During the first 10 door openings (=trials) of the first training session forelimb preference was determined and the pedestal was shifted to one side of the window to allow for reaching with the preferred limb only. At each of the two consecutive training days rats were allowed to perform 60 trials. To retrieve the pellet rats had to extend the forelimb towards the target, pronate, open the paw, grasp, and pull the forelimb back while supinating to bring the pellet towards the mouth (Whishaw & Pellis, 1990). Each reaching trial was scored as "successful" (reach, grasp and retrieve) or "unsuccessful" (pellet pushed off pedestal or dropped during retraction).

Reaching performance between sessions was measured using the success rate defined as the ratio of the number of successful trials and the total number of trials per session, i.e. 60. The CT group (n = 10) received the same pre-training like SRT rats. Pre-training was then continued for two additional sessions (equally 60 door openings on consecutive days). Thus, animals in the CT group were not required to reach outside the cage using their forelimb and were not exposed to the new motor skill. This task bears the disadvantage that changes in response to mass movements of the forelimb can hardly be differentiated from changes due learning the skilled grasp with the paw. However, a task that included gross forelimb movements also required motor learning to certain degree and induced plastic changes within M1 as shown in previous work from our group (Hosp, Mann, Wegenast-Braun, Calhoun, & Luft, 2013). To enable a sharp-cut differentiation of motor-learning related genes, we decided to choose a control paradigm that lacks an involvement of forelimb movements.

2.3. Tissue and RNA preparation

The animals were decapitated 1 h, 7 h and 24 h after the session on training day 2 (SRT group) or pre-training day 7 (CT group). At this time-point, a clear improvement in reaching performance is usually not present as the largest increase in reaching performance (i.e. "the steepest phase of the learning curve") is expected to occur between training day two and three. Thus, the processes that mediate this step are expected to occur within the 24 h after the second training session ended. To display gene-expression in this particular time-window, rats were killed at 1 h, 7 h and 24 h after day two of training. Thus, reaching performance at day three could