Neuroscience 250 (2013) 557–564

REGION AND TASK-SPECIFIC ACTIVATION OF ARC IN PRIMARY MOTOR CORTEX OF RATS FOLLOWING MOTOR SKILL LEARNING

J. A. HOSP,[a,b] S. MANN,[a,b] B. M. WEGENAST-BRAUN,[c] M. E. CALHOUN[c] AND A. R. LUFT[a,b,d,e*]

[a] *Clinical Neurorehabilitation, Department of Neurology, University of Zurich, Zurich, Switzerland*

[b] *Rehabilitation Initiative and Technology Center (RITZ), Zurich, Switzerland*

[c] *Department of Cellular Neurology, Hertie Institute for Clinical Brain Research, and DZNE, German Center for Neurodegenerative Diseases, Tübingen, Germany*

[d] *Division of Brain Injury Outcomes, Department of Neurology, Johns Hopkins University, Baltimore, MD, USA*

[e] *Center for Integrative Human Physiology, University of Zurich, Switzerland*

Abstract—Motor learning requires protein synthesis within the primary motor cortex (M1). Here, we show that the immediate early gene *Arc*/*Arg3.1* is specifically induced in M1 by learning a motor skill. *Arc* mRNA was quantified using a fluorescent *in situ* hybridization assay in adult Long–Evans rats learning a skilled reaching task (SRT), in rats performing reaching-like forelimb movement without learning (ACT) and in rats that were trained in the operant but not the motor elements of the task (controls). Apart from M1, Arc expression was assessed within the rostral motor area (RMA), primary somatosensory cortex (S1), striatum (ST) and cerebellum. In SRT animals, *Arc* mRNA levels in M1 contralateral to the trained limb were 31% higher than ipsilateral ($p < 0.001$), 31% higher than in the contralateral M1 of ACT animals ($p < 0.001$) and 48% higher than in controls ($p < 0.001$). *Arc* mRNA expression in SRT was positively correlated with learning success between two sessions ($r = 0.52$; $p = 0.026$). For RMA, S1, ST or cerebellum no significant differences in *Arc* mRNA expression were found between hemispheres or across behaviors. As *Arc* expression has been related to different forms of cellular plasticity, these findings suggest a link between M1 *Arc* expression and motor skill learning in rats.

Key words: Arc, FISH, motor learning, motor cortex.

*Corresponding author. Address: Department of Neurology, University of Zurich, Frauenklinikstrasse 26, 8091 Zurich, Switzerland. Tel: +41-44-2555400; fax: +41-44-255-4649.

E-mail address: andreas.luft@usz.ch (A. R. Luft).

Abbreviations: ACT, activity control task; ANOVA, analysis of variance; cACT, contralateral henisphere of ACT; CG, control group; cSRT, contralateral hemisphere of SRT; DIG, digoxigenin; EDTA, ethylenediaminetetraacetic acid; IEG, immediate-early gene; iSRT, ipsilateral hemisphere of SRT; LTP, long-term potentiation; M1, motor cortex; ODNs, desoxyribonucleic acids; PBS, phosphate-buffered saline; PFA, paraformaldehyde; REM, rapid eye movement; RMA, rostral motor area; ROI, regions of interests; SRT, skilled reaching task; TBS, tris-buffered saline; TEA, triethanolamine.

INTRODUCTION

While the process of motor skill learning depends on the interaction of different brain regions (e.g. sensorimotor cortex, SM; basal ganglia, BG and cerebellum, C; Hikosaka, 2002), evidence points to primary motor cortex (M1) as the structure where motor memory traces are formed. Skill acquisition requires protein synthesis within M1 and induces long lasting changes in synaptic strength (Rioult-Pedotti et al., 2000), reflecting storage mechanisms for motor memories (Kleim et al., 2003; Luft et al., 2004). However, little is known about the genes and proteins that mediate theses processes.

In rats, the immediate-early gene (IEG) c-fos is expressed within M1 after training an acrobatic locomotor skill and remains elevated when a performance plateau has been reached (Kleim et al., 1996). Recently, increased levels of Arc (activity-regulated cytoskeleton-associated protein), a protein coded by the IEG Arc (also known as the activity-regulated gene 3.1 Arg3.1) have been found in M1 of rats that trained precision reaching task with the contralateral forelimb (Hanlon et al., 2009). As this study focused on the effect of motor training on IEG expression during non-rapid eye movement (REM) sleep, it is an open question if Arc-induction was specific to learning or simply related to activity, i.e. moving the forelimb more than usually.

As IEGs have been extensively studied within the hippocampal network, all knowledge summarized here was obtained from this system unless cited differently. IEGs typically are transcribed within few minutes after induction of long-term potentiation (LTP, Worley et al., 1993; Guzowski et al., 1999). In contrast to the IEG *c-fos*, an activity-induced transcription factor that controls the expression of other transcription factors, *Arc* is an ''effector-IEG'' that promotes transcription of proteins influencing the cytoskeleton or synaptic AMPA receptor trafficking (Bramham et al., 2008; Miyashita et al., 2008). These modifications are thought to mediate learning-related cellular plasticity. Learning and experience-related transcription of *Arc* mRNA has been observed in various behavioral paradigms such as the Morris water maze task (Guzowski et al., 2000; Fletcher et al., 2006). In animals not subjected to learning paradigms, *Arc* is transcribed at very low levels. Upon excitatory synaptic activation Arc is expressed within minutes (Lyford et al., 1995) in an ''all-or-nothing'' fashion (Guzowski et al., 1999). Its induction is confined to neural assemblies associated with the encoding of

0306-4522/13 $36.00
http://dx.doi.org/10.1016/j.neuroscience.2013.06.060

information of specific behavioral experiences (Steward et al., 1998; Guzowski et al., 1999). After induction, *Arc* mRNA is transported into dendrites and accumulates at sites of synaptic activation where it is locally translated into proteins (Steward et al., 1998). Hence, Arc can be considered as a cellular marker of learning-related synaptic plasticity.

The objective here was to investigate, if Arc becomes induced in M1 and other brain regions related to motor learning in a learning-specific manner and if the degree of Arc induction is related to learning efficacy.

EXPERIMENTAL PROCEDURES

Animals

Adult male Long–Evan rats (8–10 weeks, 250–350 g, Centre d'Elevage R. Janvier, Le Genest-St. Isle, France) were used for all experiments. Animals were housed individually in a 12/12-h light/dark cycle (light on: 8 pm, off: 8 am). Littermates were distributed equally among the groups of an experiment. All experiments were conducted in accordance with German and Swiss regulations and were approved either by the Animal Commission of the State of Baden-Württemberg or the Committee for Animal Experimentation of the Canton of Zürich.

Behavioral conditions

Training sessions were performed at the beginning of the dark phase. Animals were food-restricted for 24 h before the first training session. During training animals were kept slightly over their initial weight by providing 50 mg/kg of standard lab diet after each training session. Water was given *ad libitum*. The behavioral tasks were performed as previously described (Molina-Luna et al., 2008). 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).

Three different behavioral conditions were compared: a motor skill learning paradigm (skilled reaching task; SRT), a paradigm requesting arm movements without motor learning (activity control task; ACT) and controls with the operant but without the motor elements (control group; CG). As animals from the three groups could not be evaluated in the same immunohistochemistry run for technical reasons, pairwise matching was performed between SRT/ACT and SRT/control in two runs. Animals in the SRT/ACT or SRT/CG pairings were trained for exactly the same amount of time.

As motor tasks were embedded in an operant conditioning paradigm, animals required a pre-training to operate the experimental setup properly, before being assigned to a particular experimental group. This paradigm was developed to separate the reaching trials in time to allow for a better and more precise analysis of each reach. During this pre-training, animals learned 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, Bioserve, Frenchtown, NJ, USA) located on a small horizontal board in a distance of 0.5 cm relative to the outside edge of the window. Thus, pellets could be retrieved by tongue without utilization of the forepaw. Upon retrieval a pellet dispenser automatically replaced the pellet. Whereas SRT and ACT animals were pre-trained for 5 days before being assigned to the motor task, control animals just received pre-training and were killed after the second session.

SRT

In SRT animals pre-training was followed by motor training that was initiated by removing the board and placing the pellet on a small vertical post 1.5 cm away from the window. In this position pellets were only retrievable by using the forelimb. Because the diameter of the post was approximately that of the pellet, the pellet was in an unstable position easily kicked off the post. Before the first skill training session, forelimb preference was determined. Then the pedestal was shifted to one side of the window to allow for reaching with the preferred limb only. 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 toward the mouth (Whishaw and Pellis, 1990). Each reaching trial was scored as "successful" (reach, grasp and retrieve) or "unsuccessful" (pellet pushed off pedestal or dropped during retraction). If animals missed the pellet, trials were not scored because the end of the trial was always the removal (or pushing) of the pellet from the pedestal.

For the skilled-reaching task in male Long–Evans rats, motor learning seems to be especially effective during the second day of training as the highest increase in learning success occurs between sessions two and three (Buitrago et al., 2004). Regarding the intra-session learning curve at day two, the steepest increase in successful grasps can be found between trials 40 and 60 (Buitrago et al., 2004). To display expression of *Arc* mRNA at this particular sensitive time-point, animals were killed after 50 trials at day 2 whereas training day 1 consisted of 100 trials. The improvement of reaching performance between sessions was defined as the difference of successful trials between training on day 2 (50 trials) and – to render the comparison valid – the first half (50 trials) of the training session on day 1. In case rats showed a lower performance at day 2 compared to day 1, negative values of learning rates were depicted.

ACT

The ACT consisted of extending the forelimb through the window to touch a sensor in 1.5-cm distance. If the sensor was touched, the investigator gave the rat a pellet directly into the mouth of the rat using forceps. Limb position during reaching in ACT was identical to SRT but no grasping or pellet retrieval was necessary.