Journal of Physiology - Paris 108 (2014) 263–269

Contents lists available at ScienceDirect

Journal of Physiology - Paris

journal homepage: www.elsevier.com/locate/jphysparis

Hippocampal NMDA receptors and the previous experience effect on memory

Magalí C. Cercato [1], Natalia Colettis [1], Marina Snitcofsky [1], Alejandra I. Aguirre, Edgar E. Kornisiuk, María V. Baez [2], Diana A. Jerusalinsky [2,*]

Laboratorio de Neuroplasticidad y Neurotoxinas, Instituto de Biologia Celular y Neurociencia (IBCN), Facultad de Medicina, Universidad de Buenos Aires (UBA), Paraguay 2155 3er Piso, Buenos Aires, Argentina

ARTICLE INFO

Article history:
Available online 15 August 2014

Keywords:
NMDAR
Long term memory
LTP
Open field
Inhibitory avoidance
Anti-amnesic effect
GluN2A
GluN1

ABSTRACT

N-methyl-D-aspartate receptors (NMDAR) are thought to be responsible for switching synaptic activity specific patterns into long-term changes in synaptic function and structure, which would support learning and memory. Hippocampal NMDAR blockade impairs memory consolidation in rodents, while NMDAR stimulation improves it.

Adult rats that explored twice an open field (OF) before a weak though overthreshold training in inhibitory avoidance (IA), expressed IA long-term memory in spite of the hippocampal administration of MK-801, which currently leads to amnesia.

Those processes would involve different NMDARs. The selective blockade of hippocampal GluN2B-containing NMDAR with ifenprodil after training promoted memory in an IA task when the training was weak, suggesting that this receptor negatively modulates consolidation.

In vivo, after 1 h of an OF exposure-with habituation to the environment-, there was an increase in GluN1 and GluN2A subunits in the rat hippocampus, without significant changes in GluN2B. Coincidentally, *in vitro,* in both rat hippocampal slices and neuron cultures there was an increase in GluN2A-NMDARs surface expression at 30 min; an increase in GluN1 and GluN2A levels at about 1 h after LTP induction was also shown.

We hypothesize that those changes in NMDAR composition could be involved in the "anti-amnesic effect" of the previous OF. Along certain time interval, an increase in GluN1 and GluN2A would lead to an increase in synaptic NMDARs, facilitating synaptic plasticity and memory; while then, an increase in GluN2A/GluN2B ratio could protect the synapse and the already established plasticity, perhaps saving the specific trace.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Glutamate mediates most of the excitatory neurotransmission at the central nervous system (see Paoletti et al., 2013; see Traynelis et al., 2010) by acting on both metabotropic and ionotropic receptors. The latest have been classified in KA receptor (that responds to kainic acid); AMPA receptor (AMPAR) (activated by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) and NMDA receptor (NMDAR) (that responds to N-methyl-D-aspartate). AMPAR and NMDAR are co-expressed in prefrontal cortex, temporal lobe -particularly in the hippocampus- and in other central association areas (see Paoletti et al., 2013). AMPAR supports ordinary synaptic transmission and synaptic plasticity, i.e. contributing to LTP establishment. At variance, most NMDARs are activated when postsynaptic membrane depolarization and the release of glutamate and glycine from the presynaptic side take place at the same time (Nowak et al., 1984; Wang and MacDonald, 1995), contributing to synaptic plasticity, i.e. by inducing LTP. Therefore, NMDARs are thought to be responsible for switching specific patterns of synaptic activity into long-term changes in synaptic function and structure, which would be able to support learning and memory (see Paoletti et al., 2013; see Traynelis et al., 2010).

* Corresponding author. Address: Laboratorio de Neuroplasticidad y Neurotoxinas, IBCN, Facultad de Medicina, UBA, Paraguay 2155 2nd floor, Buenos Aires (1121), Argentina. Tel.: +54 11 5950 9500x2216; fax: +54 11 5950 9626.

E-mail addresses: magacercato@yahoo.com.ar (M.C. Cercato), nataliacolettis@gmail.com (N. Colettis), marinaveterinaria@gmail.com (M. Snitcofsky), aleiaguirre@gmail.com (A.I. Aguirre), eko_rni@yahoo.com.ar (E.E. Kornisiuk), mveritobaez@gmail.com (M.V. Baez), djerusal@gmail.com (D.A. Jerusalinsky).

[1] Equal contribution to the experimental work (alphabetic order).

[2] Equivalent contribution to this paper.

http://dx.doi.org/10.1016/j.jphysparis.2014.08.001
0928-4257/© 2014 Elsevier Ltd. All rights reserved.

2. NMDAR subunits composition

NMDARs are heterotetramers composed by different subunits depending on developmental stage, neuronal activity and CNS region. There are 3 families of subunits: GluN1 (with 8 alternative splicing isoforms) (Rumbaugh et al., 2000; Vance et al., 2012), GluN2 (A, B, C and D) and, in less proportion, GluN3 (A and B). GluN1 is ubiquitously expressed through all brain regions in the adult rat since very early in development (Akazawa et al., 1994; Monyer et al., 1994; Watanabe et al., 1992), while GluN2 and GluN3 subunits show variable expression along time, development and space (Akazawa et al., 1994; Monyer et al., 1994; Sheng et al., 1994). NMDAR subunits composition changes also when neurotransmission patterns are impaired, like in stroke, epilepsy and neurodegenerative disorders (Cull-Candy and Leszkiewicz, 2004; Lacor et al., 2007; Paoletti et al., 2013; Tackenberg and Brandt, 2009; Traynelis et al., 2010).

Functional receptors are always composed by two "obligatory" GluN1 subunits and two GluN2 or GluN3 "regulatory subunits" (Cull-Candy and Leszkiewicz, 2004; see Traynelis et al., 2010). Regulatory subunits composition determines physiological and pharmacological NMDAR properties (i.e. GluN2 subunit determines Mg^{2+} affinity) (Clarke and Johnson, 2006; Dingledine et al., 1999). GluN2A and GluN2B are the predominant regulatory subunits in the hippocampus and cerebral cortex of adult animals (Akazawa et al., 1994; Monyer et al., 1994; Watanabe et al., 1992). Recently, it has been suggested that about 2/3 of hippocampal NMDARs population could be triheteromeric in rodents (Rauner and Köhr, 2011; Tovar et al., 2013).

NMDAR subunits are translated and assembled in the rough endoplasmic reticulum (RER), then transported into vesicles to the dendrites where NMDAR are inserted in the spines, sometimes directly into the synaptic membrane, probably depending on the stimulus. NMDARs are trafficked, inserted or removed from the synapse through different mechanisms depending on their subunit composition (Lavezzari et al., 2004; Tang et al., 2010; Sanz-Clemente et al., 2010; Matta et al., 2011). Based on these data there would be three different NMDAR pools: (i) the synaptic pool, (ii) the extrasynaptic pool (NMDARs in the membrane near to the synaptic membrane) and (iii) the non-synaptic pool (NMDARs present in cell body and dendrites). There is a dynamic exchange between them and their proportion seems to be related to activity (Barria and Malinow, 2002; Grosshans et al., 2002; Tovar and Westbrook, 2002; Groc et al., 2006; Bellone and Nicoll, 2007; Harris and Pettit, 2007).

2.1. Hippocampal and neocortical NMDAR composition

During embryonic development in rodents, hippocampal NMDARs are mainly composed by GluN1 and GluN2B subunits (GluN2B-NMDAR) (see Paoletti et al., 2013). Coherently, immature synapses bear mainly GluN2B-NMDAR. After birth there is an increase in GluN2A transcription and translation, while GluN2B expression remains constant from the second postnatal week; as a consequence, GluN2A/GluN2B ratio increases (Hoffmann et al., 2000). The molecular and cellular mechanisms responsible for GluN2B to GluN2A switch are not fully known, but it seems to be driven by activity. However, there are some instances, like during adolescence, when GluN2B expression and activity remains at very high levels in the prefrontal cortex. (Flores-Barrera et al., 2013; Iafrati et al., 2014).

In organotypic cultures of rat hippocampal slices, Barria and Malinow (2002) have shown that synapses undergo an activity-dependent replacement of GluN2B-NMDAR by GluN2A-NMDAR. An increase in GluN1 and GluN2A surface expression was also detected 30 min after LTP induction in hippocampal slices from adult rat, with a concomitant decrease in intracellular levels (Grosshans et al., 2002). These changes in NMDAR subunits composition were attributed to a dynamic exchange between non-synaptic and/or extra-synaptic, and the synaptic pool, since there were no changes in total NMDAR subunits level (Grosshans et al., 2002). Later on, Bellone and Nicoll (2007) reported a rapid (seconds) increase of GluN2A-NMDAR at the surface of CA1 neurons, as revealed by electrophysiological recordings after high frequency stimulation to induce LTP, in hippocampal slices from newborn mice. This increase was attributed to lateral mobility of GluN2A-NMDAR from an extrasynaptic pool. In the same model, Matta et al. (2011) have shown that the switch of GluN2 subunit composition during LTP induction depends on activity. In addition, it has recently been shown that local translation and assembling of new GluN2A-NMDAR could also be involved; these events take place at about 60 min after plasticity induction by glycine (Swanger et al., 2013), KCl pulses (Baez et al., 2013) or by NMDA (Udagawa et al., 2012).

On the other hand, it was reported that long-term depression (LTD) induction by low frequency stimulation (LFS) would require an increase in GluN2B-NMDAR and a decrease in GluN2A-NMDAR in the synaptic pool, as shown in GluN2B knock-out mice (Brigman et al., 2010) and by electrophysiological recordings in fresh slices (Dalton et al., 2012; Yu et al., 2010).

3. NMDAR, learning and memory

The NMDAR antagonist APV ((2*R*)-amino-5-phosphonovaleric acid) caused amnesia when infused *into* the hippocampus of rats trained in the Morris water maze (Morris et al., 1986; Liang et al., 1994; Packard and Teather, 1997), and also when infused into the hippocampus, amygdala or entorhinal, parietal or cingulate cortices immediately after training in other behavioral tasks (Jerusalinsky et al., 1992; Liang et al., 1994; Hlinák and Krejci, 1995; Packard and Teather, 1997; Puma and Bizot, 1998; Cammarota et al., 2004; Quinn et al., 2005). APV into the hippocampus also blocked LTP induction *in vivo* (Kim et al., 1991; Morris et al., 1986). Based on these results, Morris and colleagues postulated the LTP-NMDAR-hypothesis, which gave further support to the relationship between hippocampal LTP and spatial learning and memory (Morris and Frey, 1997; Morris et al., 2003; Kemp and Manahan-Vaughan, 2004; Eichenbaum and Fortin, 2005; Uzakov et al., 2005; Hasselmo et al., 2010), strongly stimulating further investigations in the field.

We currently assess the spontaneous and exploratory behaviour of rats in a novel open field (OF). This hippocampus dependent task can also be used to evaluate locomotion and anxiety-like behaviour (Prut and Belzung, 2003). It is expected that exploration of a new environment leads to habituation of the animal to the arena, whenever the session lasts enough to allow some trace recording/encoding for memory formation.

It is assumed that there is habituation to the OF when exploratory behaviour parameters significantly decrease intra-session, leading to short term memory (STM) and in the 2nd exposure to the arena, 24 h later, leading to long term memory (LTM).

The NMDAR antagonist APV resulted amnesic when injected into the rat hippocampus immediately after IA training (Jerusalinsky et al., 1992) and also impaired habituation after a unique OF exposure (Vianna et al., 2001). Similar results were found when hippocampal NMDAR expression was knocked-down (Cheli et al., 2002; Adrover et al., 2003; Cheli et al., 2006). These results suggest that NMDAR expression in CA1 pyramidal neurons is necessary for LTM formation of both experiences. However, the administration of MK-801, a non-competitive NMDA receptor

antagonist, during IA early consolidation though not before acquisition, caused amnesia (Jamali-Raeufy et al., 2011) when given either systemically (Venable and Kelly, 1990; Harrod et al., 2001), intraamygdala (Kim and McGaugh, 1992) or intrahippocampal (Fig. 1). Although NMDARs in the dorsal hippocampus are required during IA consolidation, GluN2B-NMDAR seems to negatively modulate this process. When hippocampal GluN2B-NMDARs were blocked with the selective antagonist Ifenprodil immediately after a weak IA training with an under-threshold stimulus (0.3 mA) which currently does not give place to LTM formation, there was expression of IA-LTM. As can be seen in Fig. 2, animals injected with Ifenprodil showed test latencies significantly higher than those injected with vehicle. Therefore, we suggest that there was a "promotion" of the trace formation and propose that GluN2B-NMDAR would act as a negative modulator during early consolidation of hippocampus-dependent memories, while GluN2A-NMDAR would either contribute to or be required for memory consolidation.

We have investigated putative changes in hippocampal NMDAR subunits expression *in vivo* after habituation to an OF (Baez et al., 2013). Adult male Wistar rats were left to freely explore an OF for 5 min, which leads to habituation to the arena; this habituation is expressed as both STM (40 min later) and LTM (24 h later) (Izquierdo et al., 1992; Vianna et al., 2001). After the OF session, rats were euthanized at times equivalent to those when changes in NMDAR subunits resulted evident in the electrophysiological assays, (Baez et al., 2013). Subunits analysis by western blot showed that both GluN1 and GluN2A levels significantly increased 70 min after a single OF trial, while GluN2B levels did not seem to change. The hippocampus of rats that were exposed for only 1 min to the OF (novelty) did not show significant changes in any of the three NMDAR subunits. Therefore, we suggest that habituation, rather than exploration or novelty, would be related to the reported changes in NMDAR subunits.

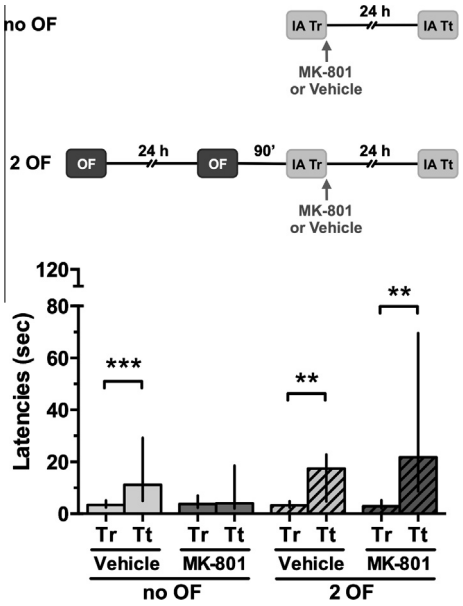

Fig. 1. Amnesia of an inhibitory avoidance task (IA) induced by MK-801 into the hippocampus "was prevented" by the previous experience in an open field (OF). Scheme on top: experimental design. Black boxes: 3 min OF sessions. Gray boxes: IA sessions (Tr: training, Tt: long-term memory test [LTM test]). Arrow: vehicle (saline) or MK-801, injected intrahippocampus immediately after IA training. Bar diagram: IA performance of rats not exposed to the OF (no OF, empty bars) or exposed to 2 OF sessions (2 OF, diagonal stripped bars). Bars represent medians of latencies with interquartile ranges (25:75). Rats injected with vehicle, either exposed or not to the OF and rats twice exposed to the OF, then injected with MK-801 after IA Tr reached the learning criterion, while those not exposed to the OF were amnesic. $^{**}p < 0.01$; $^{***}p < 0.001$, Wilcoxon paired *T* test. No OF groups: vehicle $n = 17$; MK-801 $n = 13$; 2 OF groups: vehicle $n = 13$; MK-801 $n = 17$.

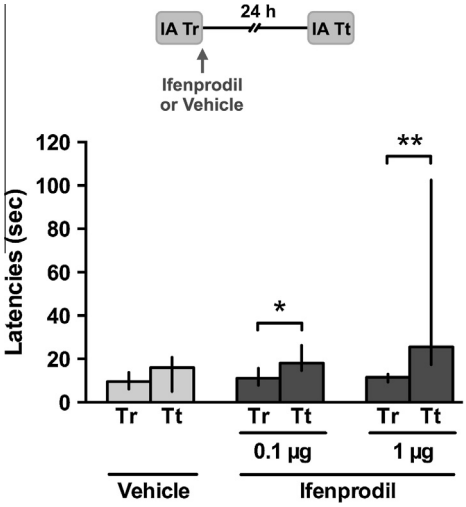

Fig. 2. Long-term memory of IA with a weak training "was promoted" by intrahippocampal administration of ifenprodil. Scheme on top: experimental design. Gray boxes: IA sessions (Tr: Training, Tt: LTM test). Arrow: intrahippocampal injection of either vehicle (DMSO in saline, 1/1000) or ifenprodil immediately after a weak training. Bar diagram: IA performance of rats injected into the dorsal hippocampus with either vehicle (light bars) or ifenprodil 0.1 and 1 µg/µl (dark bars) Bars represent medians of latencies with interquartile ranges (25:75). Ifenprodil injected groups reached the learning criterion. $^{*}p < 0.05$; $^{**}p < 0.01$, Wilcoxon paired *T* test. Vehicle $n = 16$; ifenprodil treated groups: 0.1 µg/µl, $n = 8$; 1 µg/µl, $n = 12$.

4. The "previous experience effect"

In the step-down (SD) version of IA, the adult rat is placed onto an isolated platform on one side of the training box and is left to explore it. Training latency is the time the rat takes to get down with the four paws onto the grid-floor, where it gets a mild foot-shock (Izquierdo and Ferreira, 1989; Izquierdo and Pereira, 1989; Izquierdo et al., 1999; Netto et al., 1985; Moncada and Viola, 2007, Colettis et al., 2014). Test latency is the time to get down from the platform in the test session (without foot-shock), performed 24 h later to assess long term memory (LTM). The learning criterion is reached when test latencies are significantly higher than training latencies.

Several different effects were found when animals were exposed to a new arena or to a simple behavioral task, before or after being trained in a task involving associative learning. As shown in Table 1, interaction between the OF and IA has been reported by different authors. The 1st experience (OF) could lead to interference in learning and memory of the 2nd task (IA) (Izquierdo and Ferreira, 1989; Izquierdo and Pereira, 1989); could lack of any significant effect (Izquierdo et al., 1999; Netto et al., 1985); or, there is still another possibility when the 1st task promoted encoding of a 2nd task (Moncada and Viola, 2007); i.e., OF exposure around a weak IA training session (1 h before and either 15 min or 1 h after training) with an under-threshold stimulus which would not led to LTM formation, could promote the establishment of an IA-LTM (Moncada and Viola, 2007). An OF exposure lasting 2 min, 1 h after IA training (with 0.4 or 1 mA foot-shock) or two OF exposures 5 min before and 1 h after IA training interfered with IA performance, as evidenced in the test session carried out 24 h later (Izquierdo et al., 1999). On the other hand, a shorter OF session performed 2 h before IA training had no evident effect on IA task (Netto et al., 1985). OF interference was evident when

Table 1
Effect on IA performance of a behavioral task exposure (novel experience in most cases) near to the moment of IA training.

Model	OF duration	IA shock intensity	IA Tr–Tt interval	Time interval between OF and IA Tr	Effect	References
Male rat	100 s	0.2 mA	6 h	OF 2 h after IA Tr	Θ Interference	Izquierdo and Pereira (1989)
	2 min	0.4 mA (1 mA)	0 h, 4 h, 48 h, 72 h, 96 h (for each rat)	OF 1 h after IA Tr	Θ Interference	Izquierdo et al. (1999)
				OF 6 h after IA Tr	No effect	
				OF 5 h before IA Tr (*)	No effect	
				OF 5 h before (1st) and 1 h after (2nd) IA Tr (*)	Θ Interference	
	5 min	0.15 mA	24 h	OF 2 h before IA Tr	No effect	Moncada and Viola (2007)
				OF 1 h before IA Tr	Promote LTM	
				OF 30 min before IA Tr	No effect	
				OF 15 min after IA Tr	Promote LTM	
				OF 1 h after IA Tr		
				OF 2 h after IA Tr	No effect	
Male/female rat	3 min	0.5 mA	40 min	OF 24 h (1st) and 1,5 h before (2nd) IA Tr	Overcome amnesia by IH scopolamine	Colettis et al., 2014
			24 h	OF 24 h (1st) and 1,5 h before (2nd) IA Tr	Overcome IH/i.p. scopolamine amnesia	
				OF 1.5 h before IA Tr		

Symbols: (*) Significant interference was observed when the first OF exposure (1st) lasted 2 min (5 min before IA training). No inference was observed when this first OF exposure (1st) lasted 5 min.
Abbreviations: IA: inhibitory avoidance; OF: open field; Tr. IA training session: Tt: IA test session; Θ Interference: negative interference. IH: intrahippocampal injection. i.p.: intraperitoneal injection, 1st: first OF session, 2nd: second OF session.

rats were exposed up to 2 h after IA training, but not when they were exposed 6 h after IA training (Izquierdo et al., 1999). Even in an invertebrate animal model, after a weak training protocol in which crabs did not expressed LTM, that memory could be facilitated by a single trial session (context and conditioned stimulus), whenever this takes place contingent upon the consolidation period (Smal et al., 2011). These interactions have been explained by the fact that both tasks depend, at least partially, on the same brain structure. Nevertheless, the outcome seems to depend on the order of the tasks, the interval between them, the intensity of training – including the duration of each trial – and the intrinsic timing of the encoding, associations and memory consolidation (Table 1).

Adult Wistar rats exposed to an OF for 3 or 5 min, evidenced habituation to the OF, both intra-session and in the 2nd session performed 24 h later (LTM) and compared to the 1st (Colettis et al., 2014). Therefore, we interpret that the OF is no further novel at the end of the 1st session. As mentioned in the previous section (3. NMDAR, learning and memory), we currently left the animals to freely explore twice (24 h apart) an OF for 5 min, performing the second session about 90 min before training them in different behavioral tasks. When rats exposed twice to the OF were then trained in an IA task with a mild though overthreshold foot-shock (0.5 mA), they showed an IA LTM 24 h later in spite of the administration of scopolamine into the hippocampus immediately after IA training. On the other hand, those rats treated with scopolamine that were not exposed to the OF, resulted amnesic for IA as expected (Colettis et al., 2014). When muscarinic receptor (MAChR) blockade was accomplished by intraperitoneal administration of scopolamine before IA training, animals previously exposed to the OF also expressed a LTM, showing "prevention or overcoming" of amnesia (see Section 3. NMDAR, learning and memory).

As previously mentioned, the blockade of hippocampal NMDAR by MK-801 immediately after IA training (at early consolidation) produced amnesia (Jamali-Raeufy et al., 2011). However, rats that were previously exposed twice to the OF 24 h apart, then trained in IA and injected with MK-801 immediately after training, were able to express an IA-LTM (Fig. 1). Hence, the previous OF exposure gives place to a LTM of IA in spite of the blockade of MAChRs or

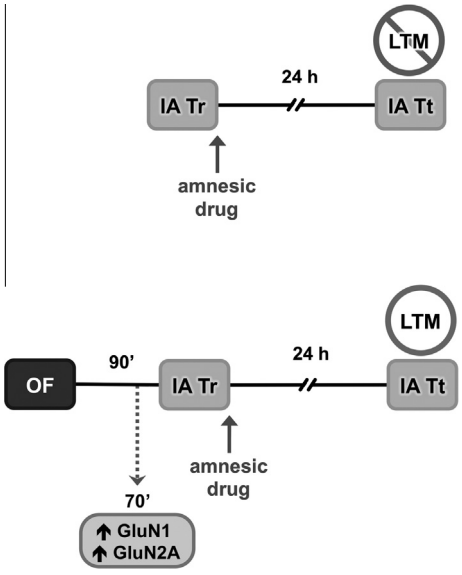

Fig. 3. Schematic representation of an "OF effect" hypothesis. Antagonists of NMDAR and MAChR (amnesic drugs) injected into the hippocampus immediately after IA training led to amnesia (top). If rats were exposed to another task, like habituation to an OF (depending at least partially on the same CNS structure), within a certain time window before IA training, the amnesia could be prevented or overcome, i.e., an IA-LTM would be expressed (bottom). As GluN1 and GluN2A NMDAR subunits increased after 70 min of the OF session (from 20 min before IA training to about 2 h later), we hypothesize that these modifications together with other synaptic plasticity factors, could be involved in "metaplasticity" or in some "synaptic tagging" induced by the OF habituation, contributing to rescue the IA trace.

NMDARs, which usually caused amnesia (Figs. 1 and 3). Interestingly, Roesler et al. (2005) have reported that the NMDAR antagonist APV injected into the dorsal hippocampus did not affect retention of an IA task in animals pre-exposed to the IA box, though APV impaired retention in rats pre-exposed to a different environment. Based on those results, the authors suggested that NMDARs in the dorsal hippocampus would mediate the contextual

representation of the task environment. However, we have shown here that the exposure to a different context, with habituation to it, also contributes to IA memory, preventing the amnesia instigated by the blockade of NMDAR in the dorsal hippocampus (Fig. 1).

The OF exposure would either promote a trace formation of IA, which could have been absent or would facilitate consolidation of an acquired trace. Our results strongly suggest that the OF would "rescue the trace" during IA consolidation (Figs. 1 and 3) and allow us to speculate that this effect appears to depend on some previous memory processing (i.e., habituation), rather than just exposure or novelty.

LTP and LTD are the main known forms of long-lasting synaptic plasticity in the CNS of vertebrates and the putative substrates for many learning and memory modalities. Most LTP and LTD require the participation of the NMDAR (Collingridge and Bliss, 1987; Lisman and McIntyre, 2001; Morris, 1989). Several studies suggested a preferential role of GluN2A for LTP and of GluN2B for LTD (Barria and Malinow, 2005; Bartlett et al., 2007; Ge et al., 2010; Massey et al., 2004; Sakimura et al., 1995). However, this hypothesis is controversial since other authors reported that GluN2B appears to be critical for LTP but not necessarily for LTD (Gardoni et al., 2009; Wang et al., 2009). These differences make it difficult to link a single NMDAR subunit with a specific form of synaptic plasticity.

Beyond the studies with pharmacological tools and transgenic animals (reviewed in Paoletti et al., 2013; Sanz-Clemente et al., 2013) to investigate the role of NMDAR subtypes in LTP and LTD, little is known about changes in expression of NMDAR subunits during synaptic plasticity induction and establishment. As already mentioned, Grosshans et al. (2002) reported an enhanced expression of GluN1 and GluN2A at the neuronal surface 30 min after LTP induction in mini-slices from adult rat hippocampus; Bellone and Nicoll (2007) found an increase in rapid currents just a few seconds after stimulation for LTP induction in slices from newborn rats. There also was an increase in dendritic expression of GluN2A 30 min after LTP induction in cultured hippocampal slices (Barria and Malinow, 2002).

Recently, we have studied GluN1, GluN2A and GluN2B hippocampal levels in slices from adult rats, after theta burst stimulation (TBS) to induce LTP. At 70 min there were significant increases of both GluN1 and GluN2A subunits, though not of GluN2B only when LTP had been effectively induced. There were not significant changes in the level of each of the three subunits 30 min after TBS (Baez et al., 2013).

As described above in Section 3, NMDAR subunits analysis in rat hippocampus showed that GluN1 and GluN2A levels significantly increased 70 min after OF exploration for 5 min. while GluN2B levels did not change.

5. Discussion and concluding remarks

NMDARs are thought to be responsible for switching synaptic activity specific patterns into long-term changes in synaptic function and structure, which would be able to support learning and memory. This receptor suffers specific changes in subunits composition that seem to be driven by (synaptic) activity, along development and along the whole life. NMDAR composition determines its physiological and pharmacological properties, being extremely relevant for circuitry activity; i.e. GluN2 subunit determines Mg^{2+} affinity (Clarke and Johnson, 2006; Dingledine et al., 1999).

During embryonic development of rodents, in the telencephalon and particularly in the hippocampus, NMDAR contains GluN1 and GluN2B subunits. After birth there is an increase in GluN2A transcription and translation that leads to an increase in GluN2A/GluN2B ratio at the synaptic membrane; as a consequence, there are more GluN2A-NMDARs than GluN2B-NMDARs in a mature synapse (see Paoletti et al., 2013; Sanz-Clemente et al., 2013). The mechanisms responsible for GluN2B to GluN2A switch along development are not fully known, though it seems to be driven by activity (Hoffmann et al., 2000; Kubota and Kitajima, 2008; Matta et al., 2011; Roberts and Ramoa, 1999), since NMDARs are trafficked, inserted or removed from the synapse through different mechanisms, depending on their subunits composition (see Lau and Zukin, 2007; see Sanz-Clemente et al., 2013; see Yashiro and Philpot, 2008).

In the adulthood, GluN2A and GluN2B are the predominant regulatory subunits in the hippocampus and cerebral cortex (Akazawa et al., 1994; Monyer et al., 1994; Watanabe et al., 1992) and the subunit composition appears to be dynamically regulated. Several authors in different models have shown that after plasticity induction, there is a rapid surface increase (in seconds to min) of GluN2A-NMDARs, and there is also a GluN2B to GluN2A switch in the membrane (Barria and Malinow, 2002; Bellone and Nicoll, 2007; Grosshans et al., 2002; Matta et al., 2011). All those NMDARs changes reported above were interpreted as dynamic exchanges between different pools.

GluN1 and GluN2A *de novo* expression increased in the adult rat hippocampus about 1 h after (1) LTP induction in slices and (2) habituation of adult rats to an OF, suggesting that NMDARs are modified in the synapse, in accordance with other authors reports (Baez et al., 2013; Barria and Malinow, 2002; Grosshans et al., 2002).

In general, it is considered that an increase in GluN2A/GluN2B ratio would contribute to synaptic maturation, including the capacity for synaptic plasticity.

The systemic blockade of NMDARs (Harrod et al., 2001; Venable and Kelly, 1990) or an antagonist infused into the hippocampus (Jamali-Raeufy et al., 2011; Jerusalinsky et al., 1992) or the amygdala (Kim and McGaugh, 1992) during IA early consolidation, though not during acquisition, produced amnesia in adult rats. Two previous OF sessions 24 h apart, prevented from the amnesia of IA caused by hippocampal NMDARs blockade (Fig. 1), as well as from the amnesia by MAChRs blockade. Since this "effect of the previous experience" took place in habituated animals we can speculate that some previous memory encoding (i.e., habituation) would be required (Colettis et al., 2014). Habituation could also be directly related to the increased expression of GluN1 and GluN2A after OF exploration (Baez et al., 2013). It is possible that in these cases, a LTM formation would require tags or other forms of metaplasticity (see Yashiro and Philpot, 2008) generated by the previous (OF) experience, which would contribute to encoding the 2nd task (IA), leading to consolidation whenever the traces of both tasks are processed (at least partially) in the same structure (sharing some circuits) (Ballarini et al., 2009; Moncada and Viola, 2007).

As reported above, we and others have shown that there were similar changes in NMDAR subunits in hippocampal slices after LTP induction and establishment (Baez et al., 2013; Barria and Malinow, 2002; Bellone and Nicoll, 2007). Furthermore, we have shown that the OF (*in vivo*) and the TBS (in hippocampal slices) substantially modified hippocampal NMDARs in the same direction, within a similar timing (Baez et al., 2013). Hence, we hypothesize that these modifications would be involved in facilitating and/or preserving synaptic plasticity and memory formation.

Altogether, these results suggest some working hypothesis: Synaptic tagging and/or metaplasticity and the related local protein synthesis at dendrites – like that of GluN2A after LTP induction by TBS and after habituation to an OF-, could be among the mechanisms involved in the rescue of a memory trace. Taking into account that the increase in GluN1 and GluN2A following OF habituation occurs from about 20 to 30 min before training in IA

and lasts for longer, this could be one of the mechanisms underlying the "anti-amnesic effect" of the previous experience. The reported GluN2A late increase into the hippocampus (at about 1 h of either the OF experience or LTP induction) could be a general feature following LTP induction/establishment, that would contribute to synaptic plasticity stabilization, i.e. by protecting the "tagged synapse" from further plasticity.

Along certain period, an increase in GluN1- and GluN2A-, would lead to a rise in membrane NMDARs underlying synaptic plasticity induction, while an increase of GluN2A/GluN2B ratio could also protect the synapse and the already established plasticity, perhaps stabilizing a specific trace during some time.

References

Adrover, M.F., Guyot-Revol, V., Cheli, V.T., Blanco, C., Vidal, R., Alché, L., Kornisiuk, E., Epstein, A.L., Jerusalinsky, D., 2003. Hippocampal infection with HSV-1-derived vectors expressing an NMDAR1 antisense modifies behavior. Genes Brain Behav. 2, 103–113.

Akazawa, C., Shigemoto, R., Bessho, Y., Nakanishi, S., Mizuno, N., 1994. Differential expression of five N-methyl-D-aspartate receptor subunit mRNAs in the cerebellum of developing and adult rats. J. Comp. Neurol. 347, 150–160.

Baez, M.V., Oberholzer, M.V., Cercato, M.C., Snitcofsky, M., Aguirre, A.I., Jerusalinsky, D.A., 2013. NMDA receptor subunits in the adult rat hippocampus undergo similar changes after 5 min in an open field and after LTP induction. PLoS One 8, e55244.

Ballarini, F., Moncada, D., Martinez, M.C., Alen, N., Viola, H., 2009. Behavioral tagging is a general mechanism of long-term memory formation. Proc. Natl. Acad. Sci. USA 106, 14599–14604.

Barria, A., Malinow, R., 2002. Subunit-specific NMDA receptor trafficking to synapses. Neuron 35, 345–353.

Barria, A., Malinow, R., 2005. NMDA receptor subunit composition controls synaptic plasticity by regulating binding to CaMKII. Neuron 48, 289–301.

Bartlett, T.E., Bannister, N.J., Collett, V.J., Dargan, S.L., Massey, P.V., Bortolotto, Z.A., Fitzjohn, S.M., Bashir, Z.I., Collingridge, G.L., Lodge, D., 2007. Differential roles of NR2A and NR2B-containing NMDA receptors in LTP and LTD in the CA1 region of two-week old rat hippocampus. Neuropharmacology 52, 60–70.

Bellone, C., Nicoll, R.A., 2007. Rapid bidirectional switching of synaptic NMDA receptors. Neuron 55, 779–785.

Brigman, J.L., Wright, T., Talani, G., Prasad-Mulcare, S., Jinde, S., Seabold, G.K., Mathur, P., Davis, M.I., Bock, R., Gustin, R.M., Colbran, R.J., Alvarez, V.A., Nakazawa, K., Delpire, E., Lovinger, D.M., Holmes, A., 2010. Loss of GluN2B-containing NMDA receptors in CA1 hippocampus and cortex impairs long-term depression, reduces dendritic spine density, and disrupts learning. J. Neurosci. 30, 4590–4600.

Cammarota, M., Barros, D.M., Vianna, M.R., Bevilaqua, L.R., Coitinho, A., Szapiro, G., Izquierdo, L.A., Medina, J.H., Izquierdo, I., 2004. The transition from memory retrieval to extinction. An Acad Bras Cienc. 76 (3), 573–582.

Cheli, V., Adrover, M., Blanco, C., Ferrari, C., Cornea, A., Pitossi, F., Epstein, A.L., Jerusalinsky, D., 2006. Knocking-down the NMDAR1 subunit in a limited amount of neurons in the rat hippocampus impairs learning. J. Neurochem. 97 (Suppl 1), 68–73.

Cheli, V.T., Adrover, M.F., Blanco, C., Rial Verde, E., Guyot-Revol, V., Vidal, R., Martin, E., Alché, L., Sanchez, G., Acerbo, M., Epstein, A.L., Jerusalinsky, D., 2002. Gene transfer of NMDAR1 subunit sequences to the rat CNS using herpes simplex virus vectors interfered with habituation. Cell. Mol. Neurobiol. 22, 303–314.

Clarke, R.J., Johnson, J.W., 2006. NMDA receptor NR2 subunit dependence of the slow component of magnesium unblock. J. Neurosci. 26, 5825–5834.

Colettis, N.C., Snitcofsky, M., Kornisiuk, E.E., Gonzalez, E.M., Quillfeldt, J.A., Jerusalinsky, D.A., 2014. Amnesia of inhibitory avoidance by scopolamine is overcome by previous open field exposure. Learn. Mem., in press.

Collingridge, G.L., Bliss, T.V.P., 1987. NMDA receptors – their role in long-term potentiation. Trends Neurosci. 10, 288–293.

Cull-Candy, S.G., Leszkiewicz, D.N., 2004. Role of distinct NMDA receptor subtypes at central synapses. Sci. STKE 2004, re16.

Dalton, G.L., Wu, D.C., Wang, Y.T., Floresco, S.B., Phillips, A.G., 2012. NMDA GluN2A and GluN2B receptors play separate roles in the induction of LTP and LTD in the amygdala and in the acquisition and extinction of conditioned fear. Neuropharmacology 62, 797–806.

Dingledine, R., Borges, K., Bowie, D., Traynelis, S.F., 1999. The glutamate receptor ion channels. Pharmacol. Rev. 51, 7–61.

Eichenbaum, H., Fortin, N.J., 2005. Bridging the gap between brain and behavior: cognitive and neural mechanisms of episodic memory. J. Exp. Anal. Behav. 84 (3), 619–629.

Flores-Barrera, E., Thomases, D.R., Heng, L.-J., Cass, D.K., Caballero, A., Tseng, K.Y., 2013. Late adolescent expression of GluN2B transmission in the prefrontal cortex is input-specific and requires postsynaptic protein kinase A and D1 dopamine receptor signaling. Biol. Psychiatry 75, 508–516.

Gardoni, F., Mauceri, D., Malinverno, M., Polli, F., Costa, C., Tozzi, A., Siliquini, S., Picconi, B., Cattabeni, F., Calabresi, P., Di Luca, M., 2009. Decreased NR2B subunit synaptic levels cause impaired long-term potentiation but not long-term depression. J. Neurosci. 29, 669–677.

Ge, Y., Dong, Z., Bagot, R.C., Howland, J.G., Phillips, A.G., Wong, T.P., Wang, Y.T., 2010. Hippocampal long-term depression is required for the consolidation of spatial memory. Proc. Natl. Acad. Sci. USA 107, 16697–16702.

Groc, L., Heine, M., Cousins, S.L., Stephenson, F.A., Lounis, B., Cognet, L., Choquet, D., 2006. NMDA receptor surface mobility depends on NR2A-2B subunits. Proc. Natl. Acad. Sci. USA 103, 18769–18774.

Grosshans, D.R., Clayton, D.A., Coultrap, S.J., Browning, M.D., 2002. LTP leads to rapid surface expression of NMDA but not AMPA receptors in adult rat CA1. Nat. Neurosci. 5, 27–33.

Harris, A.Z., Pettit, D.L., 2007. Extrasynaptic and synaptic NMDA receptors form stable and uniform pools in rat hippocampal slices. J. Physiol. 584, 509–519.

Harrod, S.B., Flint, R.W., Riccio, D.C., 2001. MK-801 induced retrieval, but not acquisition, deficits for passive avoidance conditioning. Pharmacol. Biochem. Behav. 69, 585–593.

Hasselmo, M.E., Giocomo, L.M., Brandon, M.P., Yoshida, M., 2010. Cellular dynamical mechanisms for encoding the time and place of events along spatiotemporal trajectories in episodic memory. Behav. Brain Res. 215 (2), 261–274.

Hlinák, Z., Krejci, I., 1995. Kynurenic acid and 5,7-dichlorokynurenic acids improve social and object recognition in male rats. Psychopharmacology (Berl). 120 (4), 463–469.

Hoffmann, H., Gremme, T., Hatt, H., Gottmann, K., 2000. Synaptic activity-dependent developmental regulation of NMDA receptor subunit expression in cultured neocortical neurons. J. Neurochem. 75, 1590–1599.

Iafrati, J., Orejarena, M.J., Lassalle, O., Bouamrane, L., Chavis, P., 2014. Reelin, an extracellular matrix protein linked to early onset psychiatric diseases, drives postnatal development of the prefrontal cortex via GluN2B-NMDARs and the mTOR pathway. Mol. Psychiatry 19 (4), 417–426.

Izquierdo, I., da Cunha, C., Rosat, R., Jerusalinsky, D., Ferreira, M.B., Medina, J.H., 1992. Neurotransmitter receptors involved in post-training memory processing by the amygdala, medial septum, and hippocampus of the rat. Behav. Neural Biol. 58, 16–26.

Izquierdo, I., Medina, J.H., Vianna, M.R., Izquierdo, L.A., Barros, D.M., 1999. Separate mechanisms for short- and long-term memory. Behav. Brain Res. 103, 1–11.

Izquierdo, I., Ferreira, M.B., 1989. Diazepam prevents post-training drug effects related to state dependency, but not post-training memory facilitation by epinephrine. Behav. Neural. Biol. 51 (1), 73–79.

Izquierdo, I., Pereira, M.E., 1989. Post-training memory facilitation blocks extinction but not retroactive interference. Behav. Neural Biol. 51, 108–113.

Jamali-Raeufy, N., Nasehi, M., Zarrindast, M.R., 2011. Influence of N-methyl D-aspartate receptor mechanism on WIN55, 212-2-induced amnesia in rat dorsal hippocampus. Behav. Pharmacol. 22, 645–654.

Jerusalinsky, D., Ferreira, M.B., Walz, R., Da Silva, R.C., Bianchin, M., Ruschel, A.C., Zanatta, M.S., Medina, J.H., Izquierdo, I., 1992. Amnesia by post-training infusion of glutamate receptor antagonists into the amygdala, hippocampus, and entorhinal cortex. Behav. Neural Biol. 58, 76–80.

Kemp, A., Manahan-Vaughan, D., 2004. Hippocampal long-term depression and long-term potentiation encode different aspects of novelty acquisition. Proc. Natl. Acad. Sci. USA 101 (21), 8192–8197.

Kim, J.J., DeCola, J.P., Landeira-Fernandez, J., Fanselow, M.S., 1991. N-methyl-D-aspartate receptor antagonist APV blocks acquisition but not expression of fear conditioning. Behav. Neurosci. 105 (1), 126–133.

Kim, M., McGaugh, J.L., 1992. Effects of intra-amygdala injections of NMDA receptor antagonists on acquisition and retention of inhibitory avoidance. Brain Res. 585, 35–48.

Kubota, S., Kitajima, T., 2008. A model for synaptic development regulated by NMDA receptor subunit expression. J. Comput. Neurosci. 24, 1–20.

Lacor, P.N., Buniel, M.C., Furlow, P.W., Clemente, A.S., Velasco, P.T., Wood, M., Viola, K.L., Klein, W.L., 2007. Abeta oligomer-induced aberrations in synapse composition, shape, and density provide a molecular basis for loss of connectivity in Alzheimer's disease. J. Neurosci. 27, 796–807.

Lau, C.G., Zukin, R.S., 2007. NMDA receptor trafficking in synaptic plasticity and neuropsychiatric disorders. Nat. Rev. Neurosci. 8, 413–426.

Lavezzari, G., McCallum, J., Dewey, C.M., Roche, K.W., 2004. Subunit-specific regulation of NMDA receptor endocytosis. J. Neurosci. 24, 6383–6391.

Liang, K.C., Hon, W., Davis, M., 1994. Pre- and posttraining infusion of N-methyl-D-aspartate receptor antagonists into the amygdala impair memory in an inhibitory avoidance task. Behav. Neurosci. 108 (2), 241–253.

Lisman, J.E., McIntyre, C.C., 2001. Synaptic plasticity: a molecular memory switch. Curr. Biol. 11, R788–R791.

Massey, P.V., Johnson, B.E., Moult, P.R., Auberson, Y.P., Brown, M.W., Molnar, E., Collingridge, G.L., Bashir, Z.I., 2004. Differential roles of NR2A and NR2B-containing NMDA receptors in cortical long-term potentiation and long-term depression. J. Neurosci. 24, 7821–7828.

Matta, J.A., Ashby, M.C., Sanz-Clemente, A., Roche, K.W., Isaac, J.T.R., 2011. MGluR5 and NMDA receptors drive the experience- and activity-dependent NMDA receptor NR2B to NR2A subunit switch. Neuron 70, 339–351.

Moncada, D., Viola, H., 2007. Induction of long-term memory by exposure to novelty requires protein synthesis: evidence for a behavioral tagging. J. Neurosci. 27, 7476–7481.

Monyer, H., Burnashev, N., Laurie, D.J., Sakmann, B., Seeburg, P.H., 1994. Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. Neuron 12, 529–540.

Morris, R.G., 1989. Synaptic plasticity and learning: selective impairment of learning rats and blockade of long-term potentiation in vivo by the N-methyl-D-aspartate receptor antagonist AP5. J. Neurosci. 9, 3040–3057.

Morris, R.G., Frey, U., 1997. Hippocampal synaptic plasticity: role in spatial learning or the automatic recording of attended experience? Philos. Trans. R. Soc. Lond. B Biol. Sci. 352, 1489–1503.
Morris, R.G., Hagan, J.J., Rawlins, J.N., 1986. Allocentric spatial learning by hippocampectomised rats: a further test of the "spatial mapping" and "working memory" theories of hippocampal function. Q. J. Exp. Psychol. B 38, 365–395.
Morris, R.G., Moser, E.I., Riedel, G., Martin, S.J., Sandin, J., Day, M., O'Carroll, C., 2003. Elements of a neurobiological theory of the hippocampus: the role of activity-dependent synaptic plasticity in memory. Philos. Trans. R. Soc. Lond. B Biol. Sci. 358 (1432), 773–786.
Netto, C.A., Dias, R.D., Izquierdo, I., 1985. Interaction between consecutive learnings: inhibitory avoidance and habituation. Behav. Neural Biol. 44, 515–520.
Nowak, L., Bregestovski, P., Ascher, P., Herbet, A., Prochiantz, A., 1984. Magnesium gates glutamate-activated channels in mouse central neurones. Nature 307, 462–465.
Packard, M.G., Teather, L.A., 1997. Double dissociation of hippocampal and dorsal-striatal memory systems by posttraining intracerebral injections of 2-amino-5-phosphonopentanoic acid. Behav. Neurosci. 111 (3), 543–551.
Paoletti, P., Bellone, C., Zhou, Q., 2013. NMDA receptor subunit diversity: impact on receptor properties, synaptic plasticity and disease. Nat. Rev. Neurosci. 14, 383–400.
Prut, L., Belzung, C., 2003. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. Eur. J. Pharmacol. 463, 3–33.
Puma, C., Bizot, J.C., 1998. Intraseptal infusions of a low dose of AP5, a NMDA receptor antagonist, improves memory in an object recognition task in rats. Neurosci. Lett. 248 (3), 183–186.
Quinn, J.J., Loya, F., Ma, Q.D., Fanselow, M.S., 2005. Dorsal hippocampus NMDA receptors differentially mediate trace and contextual fear conditioning. Hippocampus. 15 (5), 665–674.
Rauner, C., Köhr, G., 2011. Triheteromeric NR1/NR2A/NR2B receptors constitute the major N-methyl-D-aspartate receptor population in adult hippocampal synapses. J. Biol. Chem. 286, 7558–7566.
Roberts, E.B., Ramoa, A.S., 1999. Enhanced NR2A subunit expression and decreased NMDA receptor decay time at the onset of ocular dominance plasticity in the ferret. J. Neurophysiol. 81, 2587–2591.
Roesler, R., Reolon, G.K., Luft, T., Martins, M.R., Schröder, N., Vianna, M.R.M., Quevedo, J., 2005. NMDA receptors mediate consolidation of contextual memory in the hippocampus after context preexposure. Neurochem. Res. 30, 1407–1411.
Rumbaugh, G., Prybylowski, K., Wang, J.F., Vicini, S., 2000. Exon 5 and spermine regulate deactivation of NMDA receptor subtypes. J. Neurophysiol. 83, 1300–1306.
Sakimura, K., Kutsuwada, T., Ito, I., Manabe, T., Takayama, C., Kushiya, E., Yagi, T., Aizawa, S., Inoue, Y., Sugiyama, H., 1995. Reduced hippocampal LTP and spatial learning in mice lacking NMDA receptor epsilon 1 subunit. Nature 373, 151–155.
Sanz-Clemente, A., Gray, J.A., Ogilvie, K.A., Nicoll, R.A., Roche, K.W., 2013. Activated CaMKII couples GluN2B and casein kinase 2 to control synaptic NMDA receptors. Cell Rep. 3, 607–614.
Sanz-Clemente, A., Matta, J.A., Isaac, J.T.R., Roche, K.W., 2010. Casein kinase 2 regulates the NR2 subunit composition of synaptic NMDA receptors. Neuron 67, 984–996.
Sheng, M., Cummings, J., Roldan, L.A., Jan, Y.N., Jan, L.Y., 1994. Changing subunit composition of heteromeric NMDA receptors during development of rat cortex. Nature 368, 144–147.
Smal, L., Suárez, L.D., Delorenzi, A., 2011. Enhancement of long-term memory expression by a single trial during consolidation. Neurosci. Lett. 487 (1), 36–40.
Swanger, S.A., He, Y.A., Richter, J.D., Bassell, G.J., 2013. Dendritic GluN2A synthesis mediates activity-induced NMDA receptor insertion. J. Neurosci. 33, 8898–8908.
Tang, T.T.-T., Badger, J.D., Roche, P.A., Roche, K.W., 2010. Novel approach to probe subunit-specific contributions to N-methyl-D-aspartate (NMDA) receptor trafficking reveals a dominant role for NR2B in receptor recycling. J. Biol. Chem. 285, 20975–20981.
Tackenberg, C., Brandt, R., 2009. Divergent pathways mediate spine alterations and cell death induced by amyloid-beta, wild-type tau, and R406W tau. J. Neurosci. 29 (46), 14439–14450.
Tovar, K.R., McGinley, M.J., Westbrook, G.L., 2013. Triheteromeric NMDA receptors at hippocampal synapses. J. Neurosci. 33, 9150–9160.
Tovar, K.R., Westbrook, G.L., 2002. Mobile NMDA receptors at hippocampal synapses. Neuron 34, 255–264.
Traynelis, S.F., Wollmuth, L.P., McBain, C.J., Menniti, F.S., Vance, K.M., Ogden, K.K., Hansen, K.B., Yuan, H., Myers, S.J., Dingledine, R., 2010. Glutamate receptor ion channels: structure, regulation, and function. Pharmacol. Rev. 62, 405–496.
Udagawa, T., Swanger, S.A., Takeuchi, K., Kim, J.H., Nalavadi, V., Shin, J., Lorenz, L.J., Zukin, R.S., Bassell, G.J., Richter, J.D., 2012. Bidirectional control of mRNA translation and synaptic plasticity by the cytoplasmic polyadenylation complex. Mol. Cell 47, 253–266.
Uzakov, S., Frey, J.U., Korz, V., 2005. Reinforcement of rat hippocampal LTP by holeboard training. Learn Mem. 12 (2), 165–171.
Vance, K.M., Hansen, K.B., Traynelis, S.F., 2012. GluN1 splice variant control of GluN1/GluN2D NMDA receptors. J. Physiol. 590, 3857–3875.
Venable, N., Kelly, P.H., 1990. Effects of NMDA receptor antagonists on passive avoidance learning and retrieval in rats and mice. Psychopharmacology 100, 215–221.
Vianna, M.R., Izquierdo, L.A., Barros, D.M., de Souza, M.M., Rodrigues, C., Sant'Anna, M.K., Medina, J.H., Izquierdo, I., 2001. Pharmacological differences between memory consolidation of habituation to an open field and inhibitory avoidance learning. Braz. J. Med. Biol. Res. 34, 233–240.
Wang, D., Cui, Z., Zeng, Q., Kuang, H., Wang, L.P., Tsien, J.Z., Cao, X., 2009. Genetic enhancement of memory and long-term potentiation but not CA1 long-term depression in NR2B transgenic rats. PLoS One 4, e7486.
Wang, L.Y., MacDonald, J.F., 1995. Modulation by magnesium of the affinity of NMDA receptors for glycine in murine hippocampal neurones. J. Physiol. 486, 83–95.
Watanabe, M., Inoue, Y., Sakimura, K., Mishina, M., 1992. Developmental changes in distribution of NMDA receptor channel subunit mRNAs. NeuroReport 3, 1138–1140.
Yashiro, K., Philpot, B.D., 2008. Regulation of NMDA receptor subunit expression and its implications for LTD, LTP, and metaplasticity. Neuropharmacology 55, 1081–1094.
Yu, S.Y., Wu, D.C., Zhan, R.Z., 2010. GluN2B subunits of the NMDA receptor contribute to the AMPA receptor internalization during long-term depression in the lateral amygdala of juvenile rats. Neuroscience 171, 1102–1108.