Memory formation shaped by astroglia

Robert Zorec[1, 2*], Anemari Horvat[1], Nina Vardjan[1, 2], Alexei Verkhratsky[1, 2, 3, 4, 5, 6]

[1]Institute of Pathophysiology, University of Ljubljana, Medical Faculty, Slovenia, [2]Celica Biomedical, Slovenia, [3]Faculty of Life Sciences, University of Manchester, United Kingdom, [4]Basque Foundation for Science, Achucarro Center for Neuroscience, IKERBASQUE, Spain, [5]Department of Neurosciences, University of the Basque Country, Spain, [6]University of Nizhny Novgorod, Russia

Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

Author contribution statement

RZ, AH, NV, AV - wrote the manuscript

Keywords

astroglia, Memory, shape, Metabolism, signaling

Abstract

Word count: 195

Abstract
Astrocytes, the most heterogeneous glial cells in the central nervous system, execute a multitude of homeostatic functions and contribute to memory formation. Consolidation of synaptic and systemic memory is a prolonged process and hours are required to form long-term memory. In the past, neurons or their parts have been considered to be the exclusive cellular sites of these processes, however, it has now become evident that astrocytes provide an important and essential contribution to memory formation. Astrocytes participate in the morphological remodeling associated with synaptic plasticity, an energy-demanding process that requires mobilization of glycogen, which, in the central nervous system, is almost exclusively stored in astrocytes. Synaptic remodeling also involves bidirectional astroglial-neuronal communication supported by astroglial receptors and release of gliosignaling molecules. Astroglia exhibit cytoplasmic excitability that engages second messengers, such as Ca2+, for phasic, and cAMP, for tonic signal coordination with neuronal processes. The detection of signals by astrocytes and the release of gliosignaling molecules, in particular by vesicle-based mechanisms, occurs with a significant delay after stimulation, orders of magnitude longer than that present in stimulus–secretion coupling in neurons. These particular arrangements position astrocytes as integrators ideally tuned to support time-dependent memory formation.

Funding statement

Ethics statement

(Authors are required to state the ethical considerations of their study in the manuscript including for cases where the study was exempt from ethical approval procedures.)

Did the study presented in the manuscript involve human or animal subjects: No

3 # Memory formation shaped by astroglia

4 Robert Zorec[1,2]*, Anemari Horvat[1], Nina Vardjan[1,2], Alexej Verkhratsky[1,2,3,4,5,6]

5 [1]Laboratory of Neuroendocrinology and Molecular Cell Physiology, Institute of

6 Pathophysiology, University of Ljubljana, Faculty of Medicine, Ljubljana, Slovenia

7 [2]Celica, BIOMEDICAL, Ljubljana, Slovenia

8 [3]Faculty of Life Sciences, University of Manchester, Manchester, UK

9 [4]Achucarro Center for Neuroscience, IKERBASQUE, Basque Foundation for Science,

10 Bilbao, Spain

11 [5]Department of Neurosciences, University of the Basque Country, Leioa, Spain

12 [6]University of Nizhny Novgorod, Nizhny Novgorod, Russia

13 ***Correspondence**: Prof. Robert Zorec, Laboratory of Neuroendocrinology and Molecular

14 Cell Physiology, Institute of Pathophysiology, University of Ljubljana, Medical Faculty,

15 Zaloška cesta 4, 1000 Ljubljana, Slovenia

16 robert.zorec@mf.uni-lj.si

17 **Running title:** Astroglia Integrates Memory Formation

19

Abstract

Astrocytes, the most heterogeneous glial cells in the central nervous system, execute a multitude of homeostatic functions and contribute to memory formation. Consolidation of synaptic and systemic memory is a prolonged process and hours are required to form long-term memory. In the past, neurons or their parts have been considered to be the exclusive cellular sites of these processes, however, it has now become evident that astrocytes provide an important and essential contribution to memory formation. Astrocytes participate in the morphological remodeling associated with synaptic plasticity, an energy-demanding process that requires mobilization of glycogen, which, in the central nervous system, is almost exclusively stored in astrocytes. Synaptic remodeling also involves bidirectional astroglial-neuronal communication supported by astroglial receptors and release of gliosignaling molecules. Astroglia exhibit cytoplasmic excitability that engages second messengers, such as Ca^{2+}, for phasic, and cAMP, for tonic signal coordination with neuronal processes. The detection of signals by astrocytes and the release of gliosignaling molecules, in particular by vesicle-based mechanisms, occurs with a significant delay after stimulation, orders of magnitude longer than that present in stimulus–secretion coupling in neurons. These particular arrangements position astrocytes as integrators ideally tuned to support time-dependent memory formation.

Memory formation results in anatomical changes

Memory is the process of retention and reconstruction of learned (acquired) knowledge. Studies performed in the early 1960s on patients who underwent bilateral medial temporal lobe surgery, recognized the hippocampus as a fundamental region for memory formation (Scoville and Milner 1957). Subsequently, two distinct memory systems, declarative (explicit) memory for facts and events, for people, places, and objects ("knowing that") and non-declarative (implicit) memory, the memory for perceptual and motor skills ("knowing how"), have been defined (Dudai and Morris 2013). Both systems rely on similar, if not identical, mechanisms associated with reinforcement of synaptic transmission, which involve morphological changes at the synapse that outlast memory stabilization (Attardo et al. 2015). This morphology-based mechanism was considered by Ramon y Cajal, who linked "cerebral gymnastics" (Box 1) with morphological alterations of dendrites and terminals of neurons (Cajal 1894).

Contemporary views assume that memory formation, although it is an outcome of a myriad of interactive processes, occurs in the form of molecular events at the level of an individual synaptic connection, which is termed synaptic plasticity. These synaptic changes integrate through multiple synaptic connections involving larger neuronal networks, and are finally expressed at the behavioral level (Kandel et al. 2014).

Memory formation and astrocyte morphology

Micro-anatomical changes that are part of memory formation are not exclusively related to neurons and their parts, but involve non-neuronal cells, which in many areas of the human brain exceed the number of neurons (Azevedo et al. 2009). These non-neuronal cells include astrocytes, an abundant and arguably the most heterogeneous glial cell type in the

62   central nervous system (CNS). It is generally acknowledged that astroglia actively participate

63   in information processing via cytosolic calcium signals (Rusakov et al. 2011; Verkhratsky et

64   al. 1998).

65   A single astrocyte is intimately associated with many neurons and with their synaptic

66   contacts. A single rat cortical astrocyte enwraps 4–8 neuronal bodies and 300–600 dendrites

67   (Halassa et al. 2007), and astrocytes are in contact with synapses. In the rat hippocampus, an

68   individual astrocyte can cover (by perisynaptic processes) up to 140,000 synapses (Bushong

69   et al. 2002). Human hippocampal astrocytes are substantially larger and a single human

70   astrocyte may be associated with ~2 million synapses (Oberheim et al. 2006). Abundant

71   morphological interactions of astrocytic processes with neurons are not restricted to the

72   hippocampus, being a widespread property of CNS tissue.

73   Close morphological apposition allows astrocytes to receive signals from the synaptic

74   cleft and feedback by releasing their own signaling molecules. Release of many of these

75   molecules occurs through a secretory pathway that uses cytoplasmic vesicles, which store

76   chemical messengers. On stimulation, the vesicle membrane fuses with the plasmalemma, a

77   process termed regulated exocytosis. The role of secretory vesicles in astrocytes was proposed

78   in 1910 when Jean Nageotte suggested, based on his microscopic observations, that glial cells

79   (astroglia in particular) act as secretory elements in the CNS (Nageotte 1910). This hypothesis

80   has been confirmed experimentally in the last two decades by identifying vesicular release of

81   gliosignaling molecules, which are often termed gliotransmitters (Haydon 2001; Parpura and

82   Zorec 2010; Vesce et al. 1999; Zorec et al. 2012). Although there is some skepticism that this

83   mechanism exists in astroglia (Fujita et al. 2014; Sloan and Barres 2014), bidirectional

84   astrocyte-neuron signaling is well accepted, and it is generally recognized that vesicle-based

85   mechanisms participate in the heterocellular signaling that occurs at a morphofunctional unit

86   known as the tripartite synapse (Araque et al. 1999; Perea et al. 2009). This bidirectional

87 communication is part of the wider gliocrine system (Vardjan and Zorec 2015), which reflects

88 the secretory role of astrocytes, which release an extensive number of gliosignaling molecules

89 (Verkhratksy et al., under revision). These molecules are largely not involved in synaptic

90 processes but rather regulate various brain functions through "volume" transmission (Vardjan

91 and Zorec 2015; Zorec et al. 2015). Astroglia-derived signaling molecules are secreted into

92 the extracellular space and are transported throughout the tissue parenchyma to distant places

93 in the CNS, likely taking advantage of the glymphatic convective system (Thrane et al. 2014).

94   During implicit memory consolidation of Pavlovian threat conditioning, astrocytic

95 processes retract from synapses in the lateral amygdala, allowing these synapses to enlarge,

96 suggesting that contact with astroglial processes opposes synapse growth during memory

97 consolidation (Ostroff et al. 2014). In other words, if astrocytic processes enwrap synapses

98 and the latter need to expand during memory formation, astrocytes may hinder this

99 remodeling, demonstrating how astrocytic structural plasticity enables morphological

100 remodeling of synapses associated with memory formation. Under physiological conditions,

101 including reproduction, sensory stimulation, and learning, astrocytes display a remarkable

102 structural plasticity. Distal astrocytic processes can undergo morphological changes in a

103 matter of minutes, thus modifying the geometry and diffusion properties of the extracellular

104 space and relationships with adjacent neuronal elements, especially with synapses. This type

105 of astroglial plasticity has important functional consequences because it modifies extracellular

106 ionic homeostasis and neurotransmission, thus ultimately modulating neuronal function at the

107 cellular and system levels (Oliet and Piet 2004; Theodosis et al. 2008). The mechanisms

108 responsible for morphological changes in astrocytes are not known, but these may likely

109 involve adrenergic receptors and generation of second messenger cAMP (Vardjan et al.

110 2014), which are discussed in the following section.

Astrocyte morphology, cAMP, and metabolism

111

112   Astrocytes are capable of a remarkable morphological plasticity. Astroglial cells *in*

113 *vitro* have a flattened polygonal appearance, however stimulation of the β-adrenergic cAMP-

114 dependent signaling cascade results in rapid morphological remodeling with astrocytes

115 assuming a stellate morphology with numerous processes (Bicknell et al. 1989; Gharami and

116 Das 2004; Hatton et al. 1991; Shain et al. 1987; Shao et al. 1994; Vardjan et al. 2014; Won

117 and Oh 2000). This remodeling occurs within the time frame of memory consolidation

118 (minutes to hours) and involves cytoskeletal reorganization, including the restructuring of

119 actin filaments, microtubules, and intermediate filaments (Goldman and Abramson 1990;

120 Safavi-Abbasi et al. 2001). An example of this adrenergic receptor/cAMP-mediated

121 morphological remodeling of astrocytes is shown in Figure 1 (Vardjan et al. 2014). Similar

122 morphological plasticity may take place *in vivo* in long-term memory formation because

123 noradrenaline (NA), derived from projections of neurons located in the locus ceruleus (LC),

124 operates as a neuromodulator in Hebbian learning (Johansen et al. 2014). Under similar

125 training conditions, changes in astrocytic shape have indeed been observed (Ostroff et al.

126 2014). Moreover, the existence of structural-functional changes of the astrocyte-neuron

127 interactions during memory processes have been detected (Bernardinelli et al. 2014; Lavialle

128 et al. 2011; Perez-Alvarez et al. 2014).

129   Tight association between the synaptic membranes and astrocytes is considered

130 essential for homeostatic control of the synaptic cleft, including rapid removal of the

131 neurotransmitter glutamate (Bergles and Jahr 1997) and potassium from the extracellular

132 space (Orkand et al. 1966; Verkhratsky and Nedergaard 2014). Thus, retraction of astrocytic

133 membrane from the synapse during memory formation (Ostroff et al. 2014) may facilitate the

134 spillover of neurotransmitter and thus affect synaptic transmission (Rusakov and Kullmann

135   1998). At the same time, memory formation is associated with morphological growth of

136   synaptic elements together with enhanced protein synthesis and rearrangement of receptor

137   proteins, all of which increase the energy consumption (Harris et al. 2012).

138   How energy substrates, needed for ATP synthesis, are delivered to synapses where

139   synaptic plasticity takes place is still an open question. A simple assumption would be that

140   pyruvate is provided to the mitochondria by glycolysis within the neuron. However, the

141   morphology of astrocytes, with extensive end feet plastering blood vessels, is well suited to

142   take up glucose from blood and distribute either glucose itself, or pyruvate or lactate derived

143   from glucose, to astrocytic processes surrounding synapses, possibly by diffusion through gap

144   junctions integrating astroglial syncytia (Rouach et al. 2008). In support of this mechanism,

145   diffusion of glucose within astrocytes is relatively rapid (Kreft et al. 2013) and may well

146   support glucose delivery via interconnected astrocytes *in situ*. Although synapses are the main

147   energy consumers in the brain, glycogen, the only CNS energy storage system, is present

148   mainly, if not exclusively, in astrocytes. Memory consolidation in young chickens requires

149   glycogenolysis (Gibbs et al. 2006; Hertz and Gibbs 2009). The successful consolidation of

150   memory from short-term to long-term memory requires neuronal NA release (Gibbs et al.

151   2010). Therefore, it appears that NA, released from neurons, such as those from locus

152   coeruleus, initiates astrocytic morphological changes and activates astroglial energy

153   metabolism. Thus, NA may be considered as an integrator of the metabolism, morphology

154   and function of astrocytes. In the adult operational (i.e. awake) brain, NA is the main

155   signaling molecule that triggers astroglial Ca^{2+} signaling (Ding et al., 2013), which represents

156   the universal form of glial excitability (Verkhratsky et al., 1998).

Astrocytes as hubs for the network reset system

157

158   The LC is the primary source of NA in the CNS. It is localized in the brainstem and

159 projects widely, and is thus able to synchronously activate neural networks in several brain

160 regions. This may be regarded as a functional "reset" for many brain networks (Bouret and

161 Sara 2005; Sara 2015). Axons of LC neurons project to the spinal cord, the brain stem, the

162 cerebellum, the hypothalamus, the thalamic relay nuclei, the amygdala, the basal

163 telencephalon, and the cortex, although some cortical areas receive more abundant innervation

164 (Chandler et al. 2014). In all these structures, synchronous activation of LC projections

165 (Bouret and Sara 2005) leads to coherent and synchronized electrical activity, possibly

166 reflected by gamma waves on an electroencephalogram (Sara 2015). LC innervation mediates

167 arousal and the sleep–wake cycle, attention and memory, behavioral flexibility, behavioral

168 inhibition and stress, cognitive control, emotions, neuroplasticity, posture, and balance

169 (Benarroch 2009). The effects of NA are mediated through α- and β-adrenergic receptors

170 (α/β ARs) which are expressed in neurons, microglia, and astrocytes. The ARs were among

171 the first receptors to be causally linked to astroglial Ca^{2+} signaling (Kirischuk et al. 1996;

172 Salm and McCarthy 1989). Increases in astroglial Ca^{2+} were observed *in vivo* after stimulation

173 of the LC in anesthetized animals (Bekar et al. 2008). In awake animals, stimulation of LC

174 neurons triggered (by activation of α-ARs) widespread astroglial Ca^{2+} signals, which

175 appeared in almost all astrocytes in the field of study (Ding et al. 2013). This synchronous

176 response may represent the means by which neural networks are coordinated. Simultaneously,

177 through activation of β-ARs, the cAMP-dependent pathways are activated; this in turn

178 instigates rapid degradation of glycogen, which serves as the main energy reserve in the brain

179 (Kreft et al. 2012; Prebil et al. 2011) and initiates morphological plasticity of astrocytes

180 (Vardjan et al. 2014).

Vesicular release of gliosignaling molecules

181 By having secretory vesicles clustered close to the plasma membrane, which is a hallmark of

183 the active zone of the presynaptic terminal, the delay between the incoming stimulus and

184 secretion is minimized, being as short as 100 μs (Sabatini and Regehr 1999). At the same

185 time, vesicle-based release of chemical messengers can exhibit much longer delays in

186 stimulus–secretion coupling. In astrocytes, the mechanism prolonging the time between the

187 arrival of the stimulus and the release of transmitters has been naturally selected, because the

188 maximal speed of regulated exocytosis in astroglia appears much slower than that in neurons

189 (Guček et al. 2012; Neher 2012; Zorec et al. 2015). Regulated exocytosis also plays a role in

190 many forms of cell-to-cell communication besides release of transmitters, being for example

191 critical for the delivery of transporters, ion channels and antigen presenting molecules to the

192 cell surface (Guček et al. 2012). Vesicular traffikicng and release, which have evolved ~3

193 billion years millions years ago in arhaea (Spang et al. 2015), is fundamental for signaling and

194 communication within the relatively large eukaryotic cell volume. Communication within

195 large cells could no longer be supported by diffusion-based processes, which provide effective

196 and rapid transport of molecules within the submicron range. Hence the development of

197 subcellular organelles, such as secretory vesicles, presented a solution for the ''signaling

198 problem'' in the relatively large volume of eukaryotic cells, to which astrocytes belong

199 (Guček et al. 2012).

200   An ideal approach to monitor the rate-limiting processes of regulated exocytosis in

201 astrocytes at the cellular level is to measure changes in the plasma membrane area, which

202 reflects the fusion of vesicles with the plasma membrane. This can be monitored by

203 measuring membrane capacitance (C_m), which is linearly related to the membrane area (Neher

204 and Marty 1982). This technique was used in cultured astrocytes (Kreft et al. 2004) to test the

205 hypothesis that an increase in $[Ca^{2+}]_i$, after photolysis of caged Ca^{2+} (Neher and Zucker 1993),

206   elicits an increase in the whole-cell C_m. A half-maximal increase in C_m of these astrocytes

207   was attained at ~27 μM $[Ca^{2+}]_i$, which is similar to the Ca^{2+}-dependency of regulated

208   exocytosis in various types of neurons, recorded by a similar technique (Bollmann et al. 2000;

209   Heidelberger et al. 1994; Kreft et al. 2003a). In contrast to neurons, however, a rather small,

210   within 100 nM, increase in $[Ca^{2+}]_i$ from the resting level was sufficient to induce glutamate

211   release from astrocytes, as detected by glutamatergic effects on nearby neurons, used as

212   sniffer cells (Parpura and Haydon 2000). A similar high-affinity Ca^{2+} sensing mechanism for

213   vesicular release was reported in pituitary endocrine cells (Kreft et al. 2003b). At present,

214   astrocytes appear to be the slowest secretors of all the excitable mammalian cells investigated

215   thus far. The kinetics of C_m increase is at least two orders of magnitude slower than the

216   kinetics of regulated exocytosis recorded by a similar technique in neurons (Kreft et al. 2004;

217   Neher 2012). The Ca^{2+}-dependent increases in C_m were sensitive to tetanus toxin (which

218   cleaves synaptobrevin 2 and cellubrevin), indicating a soluble *N*-ethyl maleimide-sensitive

219   fusion protein attachment protein receptor (SNARE)-based vesicular mechanism (Kreft et al.

220   2004).

221   Why is regulated exocytosis in astrocytes so slow? One reason is the distinct slow

222   kinetics of molecular mechanisms regulating the vesicle membrane–plasmalemma merger.

223   The number of SNARE molecules per vesicle, which is relatively low in astrocytes (Singh et

224   al. 2014), may also contribute to the slow kinetics of regulated exocytosis. Slow delivery of

225   vesicles to the plasma membrane fusion sites may also play a significant role. The vesicle

226   dynamics is an amazingly elaborate system, regulated by increases in $[Ca^{2+}]_i$ (Potokar et al.

227   2013; Vardjan et al. 2015). For example, the complexity of vesicle traffic regulation in

228   astrocytes is characterized by two typical, yet opposing, properties of vesicles that contain

229   peptides, such as atrial natriuretic peptide, and/or ATP, and those that carry amino acids, such

230   as glutamate and D-serine, and are labeled by the glutamate transporter VGLUT1 (Potokar et

231   al. 2005; Potokar et al. 2013; Vardjan et al. 2012; Vardjan and Zorec 2015). Glutamatergic

232   vesicles speed up with an increase in $[Ca^{2+}]_i$ (Stenovec 2007), whereas the same increase in

233    $[Ca^{2+}]_i$ slows down peptidergic vesicles and endolysosomes (Potokar et al. 2010).

234   Glutamatergic and peptidergic vesicles have the capacity to recycle. The mobility of

235   recycling peptidergic vesicles was studied in cultured astrocytes (Potokar et al. 2008) and in

236   intact brain slices (Potokar et al. 2009). At rest, peptidergic vesicles moved faster and more

237   directionally than after the exposure of astrocytes to ionomycin to increase $[Ca^{2+}]_i$ (Potokar et

238   al. 2008). The effect of increased $[Ca^{2+}]_i$ was dramatic; the movement of vesicles was almost

239   halted, with only a jitter associated with random diffusional movement remaining. At least

240   some of the peptidergic vesicles carry ATP and a similar attenuation was observed in their

241   mobility when astrocytes were stimulated (Pangrsic 2007).

242   What is the physiologic significance of differential mobility of vesicles carrying

243   specific cargo, for example, classic chemical transmitter versus neuromodulators or

244   neuropeptides? An increase or decrease in vesicle mobility may affect the efficiency of

245   vesicle merger with the plasma membrane and the subsequent cargo discharge. It is possible

246   that vesicles engaged in the dichotomous regulation of vesicle traffic exhibit different vesicle

247   sizes, which may determine the nature of vesicle traffic and fusion with the plasmalemma, as

248   was reported for endocrine cells (Flašker et al. 2013). Increased mobility of glutamatergic

249   vesicles (which can quickly refill using VGLUTs) may indicate that they could be discharged

250   at multiple loci at times of increased Ca^{2+} excitability, resulting in more diffuse signaling as

251   opposed to spatially precise information transfer so characteristic of neuronal synaptic

252   transmission. This speculation seems to be aligned with the ability of astrocytes to modulate

253   synaptic transmission at a long temporal domain and via broad extrasynaptic access sites to

254   neurons.

255   Impaired astrocytic vesicle traffic has been tentatively associated with intellectual

256 deficiency (ID). Symptoms of ID appear early in life and the disease affects about 2% of the

257 population. Family studies have demonstrated a relatively large number of X chromosome-

258 linked forms of ID (XLID) with an incidence of about 0.9 to 1.4 in 1000 males (Turner 1996).

259 One of the first genes found to be mutated in patients with XLID is *GDI1* (D'Adamo et al.

260 1998), which encodes for guanine nucleotide dissociation inhibitor (αGDI), a protein

261 physiologically involved in regulating GDP-bound RAB proteins. The identification of *GDI1*

262 association with ID suggested that vesicular traffic in neural cells is an important pathway for

263 the development of cognitive functions (Bianchi et al. 2009; D'Adamo et al. 2002). Although

264 the importance of αGDI in neuronal function has been demonstrated, it is unclear whether its

265 role in glia vesicle trafficking contributes to the disease. The αGDI protein regulates the

266 function of RAB GTPases, such as RAB 4 and RAB 5, which have been shown to regulate

267 vesicle dynamics in astrocytes (Potokar et al. 2012), and it is likely that impaired vesicle

268 traffic in astrocytes contributes to ID, which is linked to impaired cognitive processes

269 involving memory formation.

270 ## Conclusions

271   Astroglial cells control homeostasis in the CNS to support many processes including

272 memory formation. Astrocytes contribute to memory as signaling hubs and as structures that

273 alter their morphology and recruit energy resources for memory consolidation. Excitation–

274 secretion coupling in astrocytes is loose and this may be of particular importance to support

275 the slowness of the overall memory-related structural dynamics in the CNS.

276 ## Conflict of Interest Statement

277   None declared.

Acknowledgments

References

288 Araque A, Parpura V, Sanzgiri RP, Haydon PG. 1999. Tripartite synapses: glia, the unacknowledged
289   partner. Trends Neurosci 22:208-15.
290 Attardo A, Fitzgerald JE, Schnitzer MJ. 2015. Impermanence of dendritic spines in live adult CA1
291   hippocampus. Nature 523:592-6.
292 Azevedo FA, Carvalho LR, Grinberg LT, Farfel JM, Ferretti RE, Leite RE, Jacob Filho W, Lent R,
293   Herculano-Houzel S. 2009. Equal numbers of neuronal and nonneuronal cells make the
294   human brain an isometrically scaled-up primate brain. J Comp Neurol 513:532-41.
295 Bekar LK, He W, Nedergaard M. 2008. Locus coeruleus alpha-adrenergic-mediated activation of
296   cortical astrocytes in vivo. Cereb Cortex 18:2789-95.
297 Benarroch EE. 2009. The locus ceruleus norepinephrine system: functional organization and potential
298   clinical significance. Neurology 73:1699-704.
299 Bergles DE, Jahr CE. 1997. Synaptic activation of glutamate transporters in hippocampal astrocytes.
300   Neuron 19:1297-308.
301 Bernardinelli Y, Randall J, Janett E, Nikonenko I, König S, Jones EV, Flores CE, Murai KK, Bochet CG,
302   Holtmaat A and others. 2014. Activity-dependent structural plasticity of perisynaptic
303   astrocytic domains promotes excitatory synapse stability. Curr Biol 24:1679-88.
304 Bianchi V, Farisello P, Baldelli P, Meskenaite V, Milanese M, Vecellio M, Muhlemann S, Lipp HP,
305   Bonanno G, Benfenati F and others. 2009. Cognitive impairment in Gdi1-deficient mice is
306   associated with altered synaptic vesicle pools and short-term synaptic plasticity, and can be
307   corrected by appropriate learning training. Hum Mol Genet 18:105-17.
308 Bicknell RJ, Luckman SM, Inenaga K, Mason WT, Hatton GI. 1989. Beta-adrenergic and opioid
309   receptors on pituicytes cultured from adult rat neurohypophysis: regulation of cell
310   morphology. Brain Res Bull 22:379-88.
311 Bollmann JH, Sakmann B, Borst JG. 2000. Calcium sensitivity of glutamate release in a calyx-type
312   terminal. Science 289:953-7.
313 Bouret S, Sara SJ. 2005. Network reset: a simplified overarching theory of locus coeruleus
314   noradrenaline function. Trends Neurosci 28:574-82.

315 Bushong EA, Martone ME, Jones YZ, Ellisman MH. 2002. Protoplasmic astrocytes in CA1 stratum
316   radiatum occupy separate anatomical domains. J Neurosci 22:183-92.
317 Cajal SR. 1894. The Croonian lecture: La fine structure de centres nerveux. Proc. R. Soc. Lond. p 444-
318   468.
319 Chandler DJ, Gao WJ, Waterhouse BD. 2014. Heterogeneous organization of the locus coeruleus
320   projections to prefrontal and motor cortices. Proc Natl Acad Sci U S A 111:6816-21.
321 D'Adamo P, Menegon A, Lo Nigro C, Grasso M, Gulisano M, Tamanini F, Bienvenu T, Gedeon AK,
322   Oostra B, Wu SK and others. 1998. Mutations in GDI1 are responsible for X-linked non-
323   specific mental retardation. Nat Genet 19:134-9.
324 D'Adamo P, Welzl H, Papadimitriou S, Raffaele di Barletta M, Tiveron C, Tatangelo L, Pozzi L,
325   Chapman PF, Knevett SG, Ramsay MF and others. 2002. Deletion of the mental retardation
326   gene Gdi1 impairs associative memory and alters social behavior in mice. Hum Mol Genet
327   11:2567-80.
328 Ding F, O'Donnell J, Thrane AS, Zeppenfeld D, Kang H, Xie L, Wang F, Nedergaard M. 2013. α1-
329   Adrenergic receptors mediate coordinated Ca2+ signaling of cortical astrocytes in awake,
330   behaving mice. Cell Calcium 54:387-94.
331 Dudai Y, Morris RG. 2013. Memorable trends. Neuron 80:742-50.
332 Flašker A, Jorgačevski J, Calejo AI, Kreft M, Zorec R. 2013. Vesicle size determines unitary exocytic
333   properties and their sensitivity to sphingosine. Mol Cell Endocrinol 376:136-47.
334 Fujita T, Chen MJ, Li B, Smith NA, Peng W, Sun W, Toner MJ, Kress BT, Wang L, Benraiss A and others.
335   2014. Neuronal transgene expression in dominant-negative SNARE mice. J Neurosci
336   34:16594-604.
337 Gharami K, Das S. 2004. Delayed but sustained induction of mitogen-activated protein kinase activity
338   is associated with beta-adrenergic receptor-mediated morphological differentiation of
339   astrocytes. J Neurochem 88:12-22.
340 Gibbs ME, Anderson DG, Hertz L. 2006. Inhibition of glycogenolysis in astrocytes interrupts memory
341   consolidation in young chickens. Glia 54:214-22.
342 Gibbs ME, Hutchinson DS, Summers RJ. 2010. Noradrenaline release in the locus coeruleus
343   modulates memory formation and consolidation; roles for α- and β-adrenergic receptors.
344   Neuroscience 170:1209-22.
345 Goldman JE, Abramson B. 1990. Cyclic AMP-induced shape changes of astrocytes are accompanied
346   by rapid depolymerization of actin. Brain Res 528:189-96.
347 Guček A, Vardjan N, Zorec R. 2012. Exocytosis in Astrocytes: Transmitter Release and Membrane
348   Signal Regulation. Neurochem Res.
349 Halassa MM, Fellin T, Takano H, Dong JH, Haydon PG. 2007. Synaptic islands defined by the territory
350   of a single astrocyte. J Neurosci 27:6473-7.
351 Harris JJ, Jolivet R, Attwell D. 2012. Synaptic energy use and supply. Neuron 75:762-77.
352 Hatton GI, Luckman SM, Bicknell RJ. 1991. Adrenalin activation of beta 2-adrenoceptors stimulates
353   morphological changes in astrocytes (pituicytes) cultured from adult rat neurohypophyses.
354   Brain Res Bull 26:765-9.
355 Haydon PG. 2001. GLIA: listening and talking to the synapse. Nat Rev Neurosci 2:185-93.
356 Heidelberger R, Heinemann C, Neher E, Matthews G. 1994. Calcium dependence of the rate of
357   exocytosis in a synaptic terminal. Nature 371:513-5.
358 Hertz L, Gibbs ME. 2009. What learning in day-old chickens can teach a neurochemist: focus on
359   astrocyte metabolism. J Neurochem 109 Suppl 1:10-6.
360 Johansen JP, Diaz-Mataix L, Hamanaka H, Ozawa T, Ycu E, Koivumaa J, Kumar A, Hou M, Deisseroth K,
361   Boyden ES and others. 2014. Hebbian and neuromodulatory mechanisms interact to trigger
362   associative memory formation. Proc Natl Acad Sci U S A 111:E5584-92.
363 Kandel ER, Dudai Y, Mayford MR. 2014. The molecular and systems biology of memory. Cell 157:163-
364   86.

365 Kirischuk S, Tuschick S, Verkhratsky A, Kettenmann H. 1996. Calcium signalling in mouse Bergmann
366   glial cells mediated by alpha1-adrenoreceptors and H1 histamine receptors. Eur J Neurosci
367   8:1198-208.
368 Kreft M, Bak LK, Waagepetersen HS, Schousboe A. 2012. Aspects of astrocyte energy metabolism,
369   amino acid neurotransmitter homoeostasis and metabolic compartmentation. ASN Neuro 4.
370 Kreft M, Krizaj D, Grilc S, Zorec R. 2003a. Properties of exocytotic response in vertebrate
371   photoreceptors. J Neurophysiol 90:218-25.
372 Kreft M, Kuster V, Grilc S, Rupnik M, Milisav I, Zorec R. 2003b. Synaptotagmin I increases the
373   probability of vesicle fusion at low [Ca2+] in pituitary cells. Am J Physiol Cell Physiol
374   284:C547-54.
375 Kreft M, Lukšič M, Zorec TM, Prebil M, Zorec R. 2013. Diffusion of D-glucose measured in the cytosol
376   of a single astrocyte. Cell Mol Life Sci 70:1483-92.
377 Kreft M, Stenovec M, Rupnik M, Grilc S, Krzan M, Potokar M, Pangrsic T, Haydon PG, Zorec R. 2004.
378   Properties of Ca(2+)-dependent exocytosis in cultured astrocytes. Glia 46:437-45.
379 Lavialle M, Aumann G, Anlauf E, Pröls F, Arpin M, Derouiche A. 2011. Structural plasticity of
380   perisynaptic astrocyte processes involves ezrin and metabotropic glutamate receptors. Proc
381   Natl Acad Sci U S A 108:12915-9.
382 Nageotte J. 1910. Phenomenes de secretion dans le protoplasma des cellules nevrogliques de la
383   substance grise. Paris.
384 Neher E. 2012. Introduction: regulated exocytosis. Cell Calcium 52:196-8.
385 Neher E, Marty A. 1982. Discrete changes of cell membrane capacitance observed under conditions
386   of enhanced secretion in bovine adrenal chromaffin cells. Proc Natl Acad Sci U S A 79:6712-6.
387 Neher E, Zucker RS. 1993. Multiple calcium-dependent processes related to secretion in bovine
388   chromaffin cells. Neuron 10:21-30.
389 Oberheim NA, Wang X, Goldman S, Nedergaard M. 2006. Astrocytic complexity distinguishes the
390   human brain. Trends Neurosci 29:547-53.
391 Oliet SH, Piet R. 2004. Anatomical remodelling of the supraoptic nucleus: changes in synaptic and
392   extrasynaptic transmission. J Neuroendocrinol 16:303-7.
393 Orkand RK, Nicholls JG, Kuffler SW. 1966. Effect of nerve impulses on the membrane potential of glial
394   cells in the central nervous system of amphibia. J Neurophysiol 29:788-806.
395 Ostroff LE, Manzur MK, Cain CK, Ledoux JE. 2014. Synapses lacking astrocyte appear in the amygdala
396   during consolidation of Pavlovian threat conditioning. J Comp Neurol 522:2152-63.
397 Pangrsic T. 2007. Exocytotic release of ATP from cultured astrocytes. J Biol Chem 282:28749-28758.
398 Parpura V, Haydon PG. 2000. Physiological astrocytic calcium levels stimulate glutamate release to
399   modulate adjacent neurons. Proc Natl Acad Sci USA 97:8629-8634.
400 Parpura V, Zorec R. 2010. Gliotransmission: Exocytotic release from astrocytes. Brain Res Rev 63:83-
401   92.
402 Perea G, Navarrete M, Araque A. 2009. Tripartite synapses: astrocytes process and control synaptic
403   information. Trends Neurosci 32:421-31.
404 Perez-Alvarez A, Navarrete M, Covelo A, Martin ED, Araque A. 2014. Structural and functional
405   plasticity of astrocyte processes and dendritic spine interactions. J Neurosci 34:12738-44.
406 Potokar M, Kreft M, Lee SY, Takano H, Haydon PG, Zorec R. 2009. Trafficking of astrocytic vesicles in
407   hippocampal slices. Biochem Biophys Res Commun 390:1192-6.
408 Potokar M, Kreft M, Pangrsic T, Zorec R. 2005. Vesicle mobility studied in cultured astrocytes.
409   Biochem Biophys Res Commun 329:678-83.
410 Potokar M, Lacovich V, Chowdhury HH, Kreft M, Zorec R. 2012. Rab4 and Rab5 GTPase are required
411   for directional mobility of endocytic vesicles in astrocytes. Glia 60:594-604.
412 Potokar M, Stenovec M, Gabrijel M, Li L, Kreft M, Grilc S, Pekny M, Zorec R. 2010. Intermediate
413   filaments attenuate stimulation-dependent mobility of endosomes/lysosomes in astrocytes.
414   Glia 58:1208-19.
415 Potokar M, Stenovec M, Kreft M, Kreft ME, Zorec R. 2008. Stimulation inhibits the mobility of
416   recycling peptidergic vesicles in astrocytes. Glia 56:135-44.

417 Potokar M, Vardjan N, Stenovec M, Gabrijel M, Trkov S, Jorgačevski J, Kreft M, Zorec R. 2013.
418   Astrocytic vesicle mobility in health and disease. Int J Mol Sci 14:11238-58.
419 Prebil M, Vardjan N, Jensen J, Zorec R, Kreft M. 2011. Dynamic monitoring of cytosolic glucose in
420   single astrocytes. Glia 59:903-13.
421 Rouach N, Koulakoff A, Abudara V, Willecke K, Giaume C. 2008. Astroglial metabolic networks sustain
422   hippocampal synaptic transmission. Science 322:1551-5.
423 Rusakov DA, Kullmann DM. 1998. Extrasynaptic glutamate diffusion in the hippocampus:
424   ultrastructural constraints, uptake, and receptor activation. J Neurosci 18:3158-70.
425 Rusakov DA, Zheng K, Henneberger C. 2011. Astrocytes as regulators of synaptic function: a quest for
426   the Ca2+ master key. Neuroscientist 17:513-23.
427 Sabatini BL, Regehr WG. 1999. Timing of synaptic transmission. Annu Rev Physiol 61:521-42.
428 Safavi-Abbasi S, Wolff JR, Missler M. 2001. Rapid morphological changes in astrocytes are
429   accompanied by redistribution but not by quantitative changes of cytoskeletal proteins. Glia
430   36:102-15.
431 Salm AK, McCarthy KD. 1989. Expression of beta-adrenergic receptors by astrocytes isolated from
432   adult rat cortex. Glia 2:346-52.
433 Sara SJ. 2015. Locus Coeruleus in time with the making of memories. Curr Opin Neurobiol 35:87-94.
434 Scoville WB, Milner B. 1957. Loss of recent memory after bilateral hippocampal lesions. J Neurol
435   Neurosurg Psychiatry 20:11-21.
436 Shain W, Forman DS, Madelian V, Turner JN. 1987. Morphology of astroglial cells is controlled by
437   beta-adrenergic receptors. J Cell Biol 105:2307-14.
438 Shao Y, Enkvist MO, McCarthy KD. 1994. Glutamate blocks astroglial stellation: effect of glutamate
439   uptake and volume changes. Glia 11:1-10.
440 Singh P, Jorgačevski J, Kreft M, Grubišić V, Stout RF, Potokar M, Parpura V, Zorec R. 2014. Single-
441   vesicle architecture of synaptobrevin2 in astrocytes. Nat Commun 5:3780.
442 Sloan SA, Barres BA. 2014. Looks can be deceiving: reconsidering the evidence for gliotransmission.
443   Neuron 84:1112-5.
444 Spang A, Saw JH, Jørgensen SL, Zaremba-Niedzwiedzka K, Martijn J, Lind AE, van Eijk R, Schleper C,
445   Guy L, Ettema TJ. 2015. Complex archaea that bridge the gap between prokaryotes and
446   eukaryotes. Nature 521:173-9.
447 Stenovec M. 2007. Ca2+-dependent mobility of vesicles capturing anti-VGLUT1 antibodies. Exp Cell
448   Res 313:3809-3818.
449 Theodosis DT, Poulain DA, Oliet SH. 2008. Activity-dependent structural and functional plasticity of
450   astrocyte-neuron interactions. Physiol Rev 88:983-1008.
451 Thrane AS, Rangroo Thrane V, Nedergaard M. 2014. Drowning stars: reassessing the role of
452   astrocytes in brain edema. Trends Neurosci 37:620-8.
453 Turner G. 1996. Finding genes on the X chromosome by which homo may have become sapiens. Am J
454   Hum Genet 58:1109-10.
455 Vardjan N, Gabrijel M, Potokar M, Svajger U, Kreft M, Jeras M, de Pablo Y, Faiz M, Pekny M, Zorec R.
456   2012. IFN-γ-induced increase in the mobility of MHC class II compartments in astrocytes
457   depends on intermediate filaments. J Neuroinflammation 9:144.
458 Vardjan N, Kreft M, Zorec R. 2014. Dynamics of β-adrenergic/cAMP signaling and morphological
459   changes in cultured astrocytes. Glia.
460 Vardjan N, Verkhratsky A, Zorec R. 2015. Pathologic potential of astrocytic vesicle traffic: new targets
461   to treat neurologic diseases? Cell Transplant 24:599-612.
462 Vardjan N, Zorec R. 2015. Excitable Astrocytes: Ca(2+)- and cAMP-Regulated Exocytosis. Neurochem
463   Res.
464 Verkhratsky A, Nedergaard M. 2014. Astroglial cradle in the life of the synapse. Philos Trans R Soc
465   Lond B Biol Sci 369:20130595.
466 Verkhratsky A, Orkand RK, Kettenmann H. 1998. Glial calcium: homeostasis and signaling function.
467   Physiol Rev 78:99-141.

468 Vesce S, Bezzi P, Volterra A. 1999. The active role of astrocytes in synaptic transmission. Cell Mol Life
469   Sci 56:991-1000.
470 Won CL, Oh YS. 2000. cAMP-induced stellation in primary astrocyte cultures with regional
471   heterogeneity. Brain Res 887:250-8.
472 Zorec R, Araque A, Carmignoto G, Haydon PG, Verkhratsky A, Parpura V. 2012. Astroglial excitability
473   and gliotransmission: an appraisal of Ca2+ as a signalling route. ASN Neuro 4.
474 Zorec R, Verkhratsky A, Rodríguez JJ, Parpura V. 2015. Astrocytic vesicles and gliotransmitters:
475   Slowness of vesicular release and synaptobrevin2-laden vesicle nanoarchitecture.
476   Neuroscience.
477
478

479
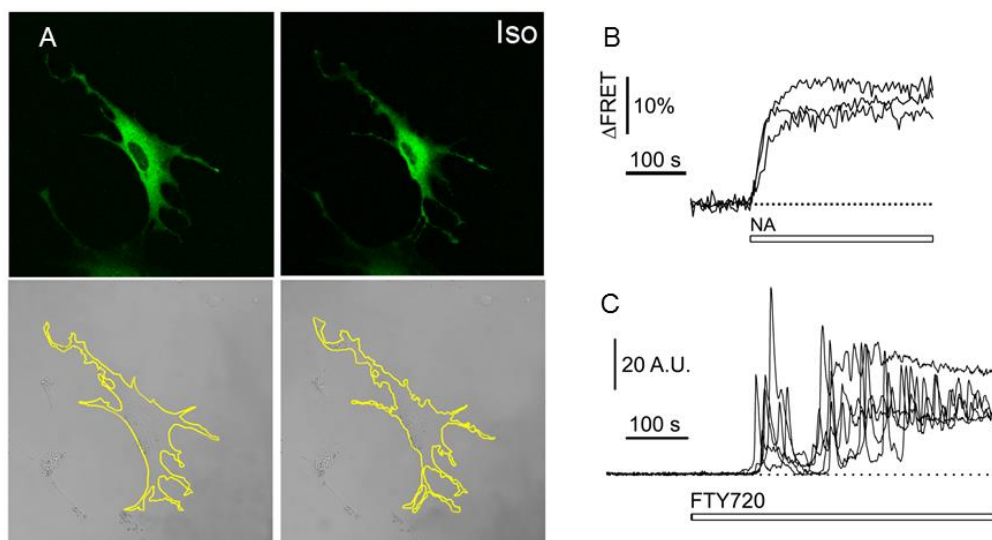
Figure 1. (A) Morphological changes in astrocytes (stellation) induced by the β-adrenergic

receptor (β-AR) agonist isoprenaline (Iso), which increases cAMP. Green fluorescing

astrocytes transfected with the cAMP nanosensor Epac1-camps (top) and their

corresponding differential interference contrast images (bottom) before (left) and after

1 μM β-AR agonist isoprenaline (Iso). Note the thinning and elongation of processes

indicating astrocyte stellation. Scale bar represents 20 μm. Astrocytes were cultured

from rat cortex. Modified from Vardjan et al. (2014) *Glia* 62, 566–579; with

permission. **(B)** Time course of cytosolic levels of cAMP. Noradrenaline (NA)

persistently increases intracellular cAMP levels in astrocytes. Representative time

courses of the Epac1-camps (i.e., a Förster resonance energy transfer (FRET)-based

cAMP nanosensor) from 3 cells after the addition of 1 μM NA. Changes in FRET are

expressed as percentages relative to the initial values. **(C)** Time course of cytosolic

levels of Ca^{2+}. The application of fingolimod (FTY720) evokes prolonged transient

increases (oscillations). Superimposed time-resolved fluorescence intensity obtained in

5 cells treated with FTY720 (white bar). The thin dotted line indicates the zero

fluorescence level (F_0). Modified from Potokar et al. (2013) *Int. J. Mol. Sci.* 14,

11238–11258; with permission.

497

Box 1. Cerebral gymnastics and memory formation

"Cerebral gymnastics are not capable of improving the organization of the brain by increasing the number of cells, because it is known that the nerve cells after the embryonic period have lost the property of proliferation; but it can be admitted as very probable that mental exercise leads to a greater development of the dendritic apparatus and of the system of axonal collaterals in the most utilized cerebral regions. In this way, associations already established among certain groups of cells would be notably reinforced by means of the multiplication of the small terminal branches of the dendritic appendages and axonal collaterals; but, in addition, completely new intercellular connections could be established thanks to the new formation of [axonal] collaterals and dendrites."

The Cronian Lecture: La fine structure des centres nerveux. *Proceedings of the Royal Society of London* 55: 444-468, 1984. Translated by DeFelipe J, Jones, E. G. (1988). *Cajal on the Cerebral Cortex*. New York: Oxford University Press. p. 87.

511