BRIEF COMMUNICATIONS

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NMDA receptor–independent long-term depression correlates with successful aging in rats

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Some individuals maintain high cognitive functioning at older ages. Here we show that mechanisms for long-term depression differ in aged rodents that maintain cognitive performance compared to young adults. Our results imply that cognitive abilities may be sustained in aged individuals by a switch in synaptic plasticity mechanisms.

On average, cognitive abilities decline with age, yet a recognizable subpopulation of individuals maintains mental abilities. At the level of neural networks, functional imaging studies have revealed that unique patterns of brain activation distinguish high-performing older individuals from younger adults[1]. Although deficits in synaptic plasticity have been identified in aged animals with cognitive impairment[2], few studies have examined the cellular basis for plasticity in aged animals with preserved cognitive abilities. Here we examined whether differences in the magnitude or the underlying mechanisms of well-characterized forms of synaptic plasticity were characteristic of high-functioning older rats.

An assessment of spatial cognition that depends on hippocampal function reveals reliable individual differences in the cognitive status of healthy aged rodents[3–5]. A previous investigation using this study population reported a significant correlation between the activity of phospholipase C (PLC) in the hippocampus and cognitive outcome across the spectrum of performance among aged rats[5]. Because PLC has been implicated in long-term depression (LTD)[6,7], we tested whether the magnitude of LTD is similarly related to cognitive performance among aged animals.

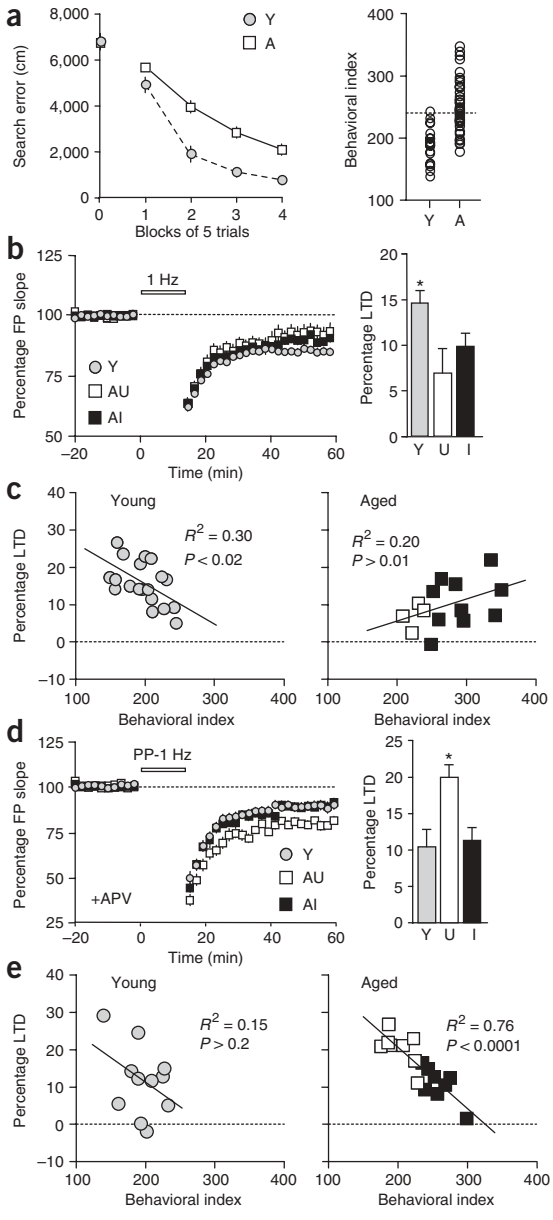

Figure 1 Switch in an LTD mechanism with age. (**a**) Aged (A) rats are impaired relative to young (Y) rats on training trials (left, $F_{1,53} = 26.12$, $P < 0.0001$) and on probe trials (right, $F_{1,53} = 36.68$, $P < 0.0001$). Note there is no difference in initial performance (left panel, first data point). Right panel shows behavioral index in probe trials. Dotted line indicates the cut-off at 240 for dividing aged rats into aged-unimpaired (AU) and aged-impaired (AI) groups. All behavioral scores derive from a proximity measure (distance from platform sampled 10 times per s). High values reflect less accurate search on training trials (search error) and probe trials (behavioral index) (see **Supplementary Methods** online). (**b**) Larger NMDAR-LTD in Y compared to AU and AI. Right, comparison of average LTD magnitude during the last 10 min. (**c**) NMDAR-LTD magnitude correlates with the behavioral index in young, but not aged, rats. (**d**) Non-NMDAR-LTD is larger is AU compared to Y and AI. Right, comparison of the non-NMDAR-LTD magnitude. (**e**) Non-NMDAR-LTD magnitude correlates with the behavioral index in aged, but not young, animals. $^*P < 0.02$, Fisher's protected least squares difference (PLSD) post-hoc analysis. Gray circles, Y; white squares, AU; black squares, AI. Error bars indicate s.e.m.

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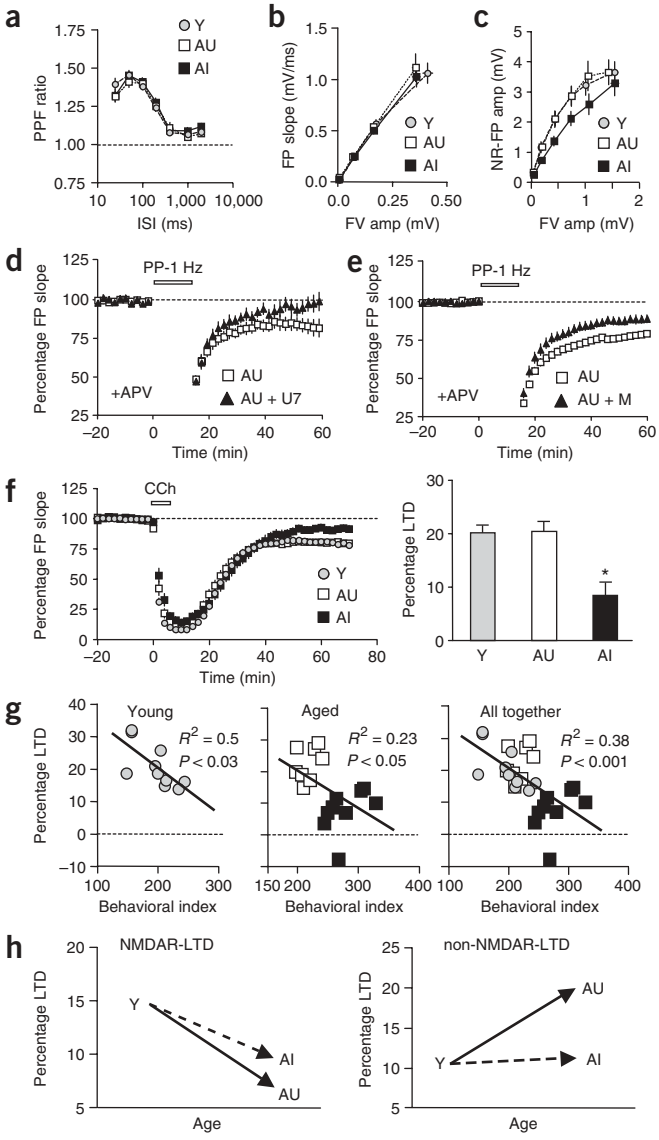

Figure 2 Basal synaptic transmission and PLC dependence of LTD. (**a**) Similar paired-pulse facilitation (PPF) ratio across different groups. (**b**) No difference in AMPAR–mediated basal synaptic transmission. Field potential (FP) slopes were plotted against the presynaptic fiber volley (FV) amplitudes. (**c**) Pharmacologically isolated NMDAR–mediated synaptic transmission (by 10 μM NBQX and 0 mM $MgCl_2$) was less in AI, but not different between Y and AU. FP amplitudes were plotted against the FV amplitudes. (**d**) Non-NMDAR-LTD in AU is blocked by a PLC inhibitor (U7: 10 μM U73122). (**e**) Non-NMDAR-LTD in AU is significantly reduced ($P < 0.003$) by bath application of an inhibitor cocktail (M: 10 μM MPEP, 10 μM prazosin and 5 μM atropin, antagonists of mGluR5, α1-adrenergic receptor and M1-mAchR, respectively). (**f**) Carbachol (CCh)-induced LTD is less in AI compared to AU and Y animals. Right, comparison of the average CCh-LTD magnitude. (**g**) CCh-LTD magnitude correlates with the behavioral index across ages. (**h**) Age-dependent switch in an LTD mechanism. NMDAR-LTD declines with age (left), whereas non-NMDAR-LTD increases in AU rats (right). *$P < 0.001$, Fisher's PLSD post-hoc analysis. Gray circles, Y; white squares, AU; black squares, AI. Error bars indicate s.e.m.

In this study, we measured LTD in area CA1 of hippocampal slices prepared from behaviorally characterized young (6 months of age) and aged (24 months of age) outbred Long-Evans rats (**Fig. 1**). The aged rats included a substantial subset that performed within the range of young adult rats in a hippocampal-dependent assessment of spatial learning (**Fig. 1a**, right panel). These aged-unimpaired (AU) rats were compared to both young adults (Y) and aged rats that had deficits in the behavioral assessment (AI: aged-impaired). First we measured LTD induced with a standard 1 Hz, 15 min protocol (a train of current pulses of 0.2 ms duration), which produces an NMDA receptor–dependent LTD (NMDAR-LTD, **Supplementary Fig. 1** online)[8]. Young animals had, on average, larger NMDAR-LTD than the aged animals, but there was no difference between the AU and AI groups (**Fig. 1b**) (at 1 hr after onset of 1 Hz, mean NMDAR-LTD ± s.e.m., relative to baseline, was as follows: Y: 86 ± 1.5%, $n = 63$ slices, 20 rats; AU: 94 ± 2.7%, $n = 16$ slices, 4 rats; AI: 90 ± 1.5%, $n = 39$ slices, 10 rats; ANOVA: $F_{2,115} = 5.497$, $P < 0.006$). More importantly, we found that the average magnitude of NMDAR-LTD per animal correlated significantly with the behavioral index only in young, but not in aged, rats (**Fig. 1c**).

Next we examined an NMDAR–independent form of LTD (non-NMDAR-LTD), which was induced by delivering paired pulses (50-ms interstimulus interval) repeated at 1 Hz for 15 min (PP-1Hz) in the presence of a blocker of NMDAR (100 μM DL-2-amino-5-phosphonopentanoic acid, DL-APV)[9]. The AU group showed a significantly larger non-NMDAR-LTD than either the young or the AI groups (Y: 90 ± 2.2%, $n = 33$ slices, 11 rats; AU: 79 ± 2.2%, $n = 22$ slices, 8 rats; AI: 87 ± 2.1%, $n = 20$ slices, 8 rats; ANOVA: $F_{2,72} = 5.917$, $P < 0.005$; **Fig. 1d**). Moreover, we found a significant correlation between the magnitude of non-NMDAR-LTD and the behavioral index in aged, but not in young, animals (**Fig. 1e**). Collectively, these results suggest that the behavioral index correlates with the magnitude of NMDAR-LTD in young animals and with that of non-NMDAR-LTD in aged animals.

We then examined whether the differences in LTD mechanisms in young and aged rats could be due to changes in basal synaptic transmission. Paired-pulse facilitation ratios and input-output curves, which respectively reflect presynaptic function and AMPA receptor–mediated basal synaptic transmission, were similar between the three groups (**Fig. 2a**,**b**). The pharmacologically isolated NMDAR-mediated synaptic responses were slightly reduced in AI rats. However, there was no difference between young and AU animals (**Fig. 2c**), suggesting that alterations in NMDAR responses cannot account for the reduced NMDAR-LTD in AU animals.

Non-NMDAR-LTD in AU was blocked by a specific inhibitor of PLC, U73122 (**Fig. 2d**), and was attenuated by combined antagonists for PLC-linked receptors (**Fig. 2e**). Application of single receptor antagonists had no effect (data not shown). Similar to a previous report[6], NMDAR-LTD in young rats was also blocked by U73122 (**Supplementary Fig. 1**). The dependence of LTD on PLC activity prompted us to examine whether the magnitude of other forms of PLC-dependent LTD, such as carbachol (CCh)-induced LTD[7,10], also changes with age. We found that the average magnitude of CCh-LTD was significantly less in AI rats than in young and AU rats (Y: 78 ± 1.5% at 70 min post-CCh, $n = 59$ slices, 10 rats; AU: 80 ± 1.9%, $n = 33$ slices, 8 rats; AI: 92 ± 2.8%, $n = 34$ slices, 10 rats; ANOVA: $F_{2,125} = 14.559$, $P < 0.01$; **Fig. 2f**). Moreover, the magnitude of CCh-LTD correlated with the behavioral index across ages (**Fig. 2g**).

Our results show that behavioral performance correlates with NMDAR-LTD in young animals and with non-NMDAR-LTD in aged animals. NMDAR-LTD is reduced in aged rats, but unimpaired animals seem to show an increase in non-NMDAR-LTD (**Fig. 1d**,**e**). This suggests that high-functioning aged rats maintain the ability to generate LTD, but do so by different mechanisms than those used by young

adults (**Fig. 2h**). It remains to be determined whether the diminished NMDAR-LTD in aged animals may have resulted from a shift in the activity requirement for the recruitment of NMDARs or from a downregulation of the machinery that couples NMDARs to LTD. In any case, our data imply that the PLC signaling pathway is activated via NMDARs in young rats (**Supplementary Fig. 1**), but predominantly via other receptors in AU rats (**Fig. 2d**), suggesting that aged animals that fail to make this switch will show impaired performance.

NMDAR-LTD has been reported previously to increase with age[8], which is seemingly at odds with our data showing a decrease with age (**Fig. 1b**,**c**). The difference may be due to the strain of rats used (Long-Evans rats versus Fischer 344 rats[8]). Nonetheless, the magnitude of NMDAR-LTD correlated favorably with the behavioral index only in young adults but not in aged animals. Indeed, an opposite trend among the aged rats indicated that the worst performers tended to have slightly larger NMDAR-LTD (**Fig. 1c**), consistent with an association between NMDAR-LTD and impairment in older animals.

Notably, our findings show that age-related cognitive impairment may be rescued by switching from an NMDAR-dependent to a non-NMDAR-dependent LTD (**Fig. 2h**). We further speculate that this switch has a neuroprotective function. Many studies show that excessive NMDAR activation leads to excitotoxicity[11,12], and this signaling is due to the association of a macromolecular signaling complex to the receptor[13]. A dissociation of the macromolecular signaling complex can effectively uncouple NMDAR activation and recruitment of downstream effectors[14]. Similar uncoupling in AU rats would be consistent with a reduced NMDAR-LTD without a change in the NMDAR responses. We propose that this type of uncoupling has consequences for preventing cognitive decline with age. It will be of future interest to test this possibility and determine the factors that mediate an effective switch in LTD mechanisms that may promote successful aging.

Note: Supplementary information is available on the Nature Neuroscience website.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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1. Cabeza, R. *Psychol. Aging* **17**, 85–100 (2002).
2. Barnes, C.A. *Phil. Trans. R. Soc. Lond. B* **358**, 765–772 (2003).
3. Gallagher, M., Burwell, R. & Burchinal, M. *Behav. Neurosci.* **107**, 618–626 (1993).
4. Nicholson, D.A., Yoshida, R., Berry, R.W., Gallagher, M. & Geinisman, Y. *J. Neurosci.* **24**, 7648–7653 (2004).
5. Nicolle, M.M., Colombo, P.J., Gallagher, M. & McKinney, M. *J. Neurosci.* **19**, 9604–9610 (1999).
6. Reyes-Harde, M. & Stanton, P.K. *Neurosci. Lett.* **252**, 155–158 (1998).
7. Kirkwood, A., Rozas, C., Kirkwood, J., Perez, F. & Bear, M.F. *J. Neurosci.* **19**, 1599–1609 (1999).
8. Norris, C.M., Korol, D.L. & Foster, T.C. *J. Neurosci.* **16**, 5382–5392 (1996).
9. Huber, K.M., Roder, J.C. & Bear, M.F. *J. Neurophysiol.* **86**, 321–325 (2001).
10. Auerbach, J.M. & Segal, M. *J. Physiol. (Lond.)* **492**, 479–493 (1996).
11. Arundine, M. & Tymianski, M. *Cell. Mol. Life Sci.* **61**, 657–668 (2004).
12. Lynch, D.R. & Guttmann, R.P. *J. Pharmacol. Exp. Ther.* **300**, 717–723 (2002).
13. Husi, H., Ward, M.A., Choudhary, J.S., Blackstock, W.P. & Grant, S.G. *Nat. Neurosci.* **3**, 661–669 (2000).
14. Sattler, R. *et al. Science* **284**, 1845–1848 (1999).