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Changing roles of NMDA receptor subtypes in synaptic plasticity during development in perirhinal cortex *in vitro*

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It is widely believed that synaptic plasticity may be the underlying process behind learning and memory. One form of plasticity, long-term depression (LTD), is known to be down-regulated during development, though the purpose of this remains unclear. During development, it has been shown that there is a relative increase in the expression of type 2A NMDA receptors relative to 2B, and that these receptors are targeted to the synapse. We have previously demonstrated that LTD in adult perirhinal cortex is primarily mediated by extrasynaptic NR2B receptors, while LTP and depotentiation are mediated by NR2A receptors (Massey et al. 2004). In this report, we have investigated further the developmental roles of these NMDA receptor subtypes. Perirhinal cortex slices were prepared from humanely killed juvenile (P14) rats. Extracellular field recordings were made from layer II/III in response to stimuli delivered to either side of the recording electrode. LTD was induced using the low-frequency stimulation (LFS, 900 stimuli at 1Hz) protocol (70 ± 9% of baseline, n=6). Application of the NMDA receptor antagonist AP5 (50 μM) blocked this LTD (103 ± 18%, n=3). Antagonists of NR2A and NR2B receptors were then applied during LTD induction to determine subtype specificity. Ro 25-6981 (3 μM), a NR2B inhibitor, had a small effect upon LTD (81 ± 3%, n=4) whereas the NR2A inhibitor NVP-AAM077 (0.5 μM) blocked the majority of LTD (93 ± 3%, n=5). These results, when compared with findings in adults, suggest that there are changes during development in the role of these two NMDA receptor subtypes in mediating synaptic plasticity.

Massey et al. (2004). J Neurosci 24, 7821-7828.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C110

Developmental changes in spontaneous glutamate release and its modulation by metabotropic glutamate receptors in the rat entorhinal cortex

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We have previously demonstrated that activation of group III metabotropic glutamate receptors (mGluRs) facilitates spontaneous glutamate release in rat entorhinal cortex (EC) in vitro (Evans et al. 2000), and group II mGluRs have a similar effect (Ayman et al. 2003). This uncharacteristic effect of group II and group III mGluRs was recorded in slices prepared from young animals (4-6 weeks). Since developmental changes in expression and function of these receptors have been demonstrated (e.g. Ross et al. 2000), it was of interest to determine if there were changes in the effects of the mGluRs in the rat EC with age.

Slices of EC were prepared from humanely killed male Wistar rats aged 4-6 weeks or 5-6 months. Glutamate release was monitored by recording spontaneous excitatory postsynaptic currents (sEPSCs) from neurones in layer V. All recordings were made in the presence of the NMDA receptor blocker, MK-801 (10 μM), so EPSCs reflect activation of AMPA receptors. Statistical comparisons were made using either the Wilcoxon rank sum test (WRST) of the Kolmogorov-Smirnoff test (KS).

Mean interevent interval (IEI) for sEPSCs recorded from 16 cells in the young group was 312.5±7.1 ms. Whereas, in the older animals mean IEI (n = 14) it was 21 % less at 246.5±6.6 ms ($P < 0.05$, WRST). The mean amplitude of events was 13.4±0.2 pA for the older group and 11.1±0.1 pA for the young group ($P < 0.05$, WRST). In the case of miniature (m)EPSCs, recorded in TTX, mean IEI was over 200% greater in the young group (896.4±32.9 ms, n = 7) than in the older animals (287.6±10.8 ms, n = 4; $P < 0.05$, WRST). Thus, in the older animals, EPSCs were larger, more frequent and have a smaller contribution from action potential-driven release.

The effects of the group III mGluR agonist, ACPT-1, were tested on 8 layer V neurones recorded in slices from older animals. Mean IEI under control conditions was 265.7±9.5 ms and mean amplitude was 12.6±0.2 pA. In the presence of ACPT-1 (20 μM), mean IEI increased to 357.3±10.3 ms ($P < 0.001$, KS). Mean amplitude was unchanged. Subsequent addition of DCG-IV (5 μM), a group II mGluR agonist, increased IEI further to 520.8±15.1 ms ($P < 0.001$, KS), without change in amplitude.

Thus, in addition to the overt changes in spontaneous glutamate release seen in the older animals, there was a reversal of the effects of group II and group III mGluRs, both now eliciting a depression of release in contrast to the facilitation seen in younger animals. Whether this reflects developmental differences in the receptors per se, or whether it is due to intrinsic changes in the release mechanism is a subject for further investigation.

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Ayman G *et al.* (2003). *Brit J Pharm* **138**, 191P.

Ross FM *et al.* (2000). *Brit J Pharmacol* **131**, 453-464.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C112

Injections of urocortin 1 into basolateral nucleus of the amygdala induce anxiety-like behaviour and c-Fos expression in topographically organized subpopulations of serotonergic neurons

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The amygdala plays a key role in emotional processing and anxiety responses. Previous studies have shown that urocortin 1 (Ucn 1) injections into the basolateral nucleus of the amygdala (BLA) induce anxiety-like behavior. Brainstem serotonergic systems in the dorsal raphe nucleus (DR) may be part of a distributed neural system

that, together with the BLA, regulates acute and chronic anxiety states. We therefore investigated the effect of an acute bilateral injection of Ucn 1 into the BLA on behavior in the social interaction test (SIT) and on c-Fos expression within serotonergic neurons in the DR. Male rats were anaesthetized with a combination of Diazepam (0.2 ml, I.P.) and Hypnorm (0.2 ml, I.M.), and implanted with bilateral cannulae into the BLA; 72 h after surgery, rats were injected with Ucn 1 (50 fmol/100 nl) or vehicle (100 nl of 1% bovine serum albumin in distilled water). Thirty minutes after injection, a subgroup from each experimental group was exposed to the SIT; remaining animals were left in the home cage. Two hours after injection rats were anesthetized with 0.5 ml Euthatal (200 mg/ml sodium pentobarbital, I.P.), perfused with paraformaldehyde and brains were removed and processed for immunohistochemistry.

Acute injection of Ucn 1 reduced total interaction time in the social interaction paradigm without affecting locomotor activity or exploratory behavior. Ucn 1 treatment induced c-Fos immunoreactivity in serotonergic neurons within the dorsal part of the rostral DR (- 7.46 mm bregma) in home cage rats, and within the dorsal and ventral parts of the middle and caudal DR (-8.00 and -8.54 mm bregma, respectively) in rats exposed to the SIT. These results are consistent with the hypothesis that the BLA and topographically organized subpopulations of serotonergic neurons within the DR are part of an integrated neural system modulating anxiety state.

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The perirhinal cortex as part of the (para)hippocampal memory system. A connectionists perspective on function

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The perirhinal cortex is part of a network of cortices in the medial temporal lobe that have been suggested to be involved in declarative memory processes. Together with the parahippocampal or postrhinal cortex and the entorhinal cortex, the perirhinal cortex constitutes a major input/output module, mediating information transfer between the hippocampus and widespread areas of the cortex. Interestingly, the cortical connections of the perirhinal cortex appear to be different from those of the parahippocampal/postrhinal cortex, suggesting a functional differentiation. In the lecture, I will summarize the specific organization of perirhinal-entorhinal-hippocampal connections, relate this to perirhinal-cortical connectivity, and compare this with the connectivity of the parahippocampal/postrhinal cortex.

Findings in both non-human primates as well as in rodents converge onto the notion that a possible functional differentiation between the perirhinal cortex and the parahippocampal/postrhinal cortex relates to different portions of the entorhinal cortex, as well as to different hippocampal domains. Therefore, the data appear to indicate that the cortico-parahippocampal-hippocampal system comprises at least two parallel routes, which mediate the transfer of functionally different types of information. These two parallel routes are organized such that cross-talk, i.e. convergence, may occur at various hierarchical stages of processing.

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SA50

Age- and experience-dependent modification of LTD in the perirhinal cortex

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Perirhinal cortex is association cortex that has been shown to be critically involved in visual recognition memory, and loss of recognition memory is a major symptom of the amnesic syndrome and early Alzheimer's disease (Brown & Aggleton, 2001). There is good evidence that the familiarity discrimination component of recognition memory depends on reductions in responses of perirhinal neurones (Warburton et al. 2003). Therefore, it is thought that a potential cellular mechanism of encoding this form of memory is through synaptic plasticity, such as long-term depression (LTD) (Brown & Bashir, 2002; Warburton et al. 2003).

Mechanisms of synaptic plasticity, such as LTP and LTD, are thought to underlie development of the CNS and learning and memory. Synaptic plasticity is known to be developmentally regulated in many brain regions and the changes in mechanisms that occur during development are a matter of intense investigation (Kemp & Bashir 2001; Philpot et al. 2001).

In the present study, we demonstrate that during development of the perirhinal cortex LTD switches from being metabotropic glutamate (mGlu) receptor-dependent to being muscarinic acetylcholine receptor-dependent. Importantly, this switch occurs as a consequence of visual experience and suggests that changing physiological functions require or produce different forms of synaptic plasticity.

Slices of perirhinal cortex were prepared from neonatal (7–12 day old) and adult (28–35 day old) Wistar rats. All efforts were made to minimize the numbers of animals used. Animals were humanely killed. Either standard whole-cell or field recordings were used during this study. Data for any set of experiments were only analysed from one slice per rat (n). Data pooled across slices are expressed as the mean ± SEM and significance ($P\langle 0.05$).

5 Hz stimulation delivered to neonatal (P7–12) perirhinal cortex slices resulted in robust NMDA receptor-independent homosynaptic LTD (29 ± 7%, n=7). To minimise any possible confounding contribution of NMDA receptor activation under different conditions, all subsequent experiments were carried out in the presence of 50μM AP5.

In many other studies it has been shown that LTD that is NMDA receptor-independent is mGluR-dependent (Kemp & Bashir. 2001). In keeping with this, the mGluR5 receptor antagonist MPEP (10μM), blocked LTD in a reversible manner in P7–12 perirhinal cortex (5 ± 1% depression in MPEP; 18 ± 3% depression after washout of MPEP, n=7). In contrast, however, neither group II nor group III mGlu receptor antagonists (200μM EGLU and 100nM CPPG, respectively) had any effect on LTD (25 ± 1%, n=5, EGLU; 22 ± 2%, n=4, CPPG). 5 Hz stimulation also resulted in NMDA receptor-independent LTD in adult (P28–35) perirhinal cortex (18 ± 1%, n=5). However, in contrast to LTD in P7–12 animals, LTD was not mGlu5 receptor-dependent; the mGlu5 antagonist MPEP had no effect on 5 Hz-LTD in adult cortex (18

± 2% LTD, n=6). Therefore, LTD in the adult cortex relies on cellular mechanisms different to LTD in P7–12 cortex. In the present study we find that inhibition of muscarinic receptors blocked 5 Hz-LTD in P28–35 perirhinal cortex (2 ± 3% in 20μM scopolamine; n=6). In contrast to these results, the muscarinic receptor antagonist scopolamine did not prevent the induction of LTD in P7-12 perirhinal cortex (32 ± 5% in scopolamine, n=6).
Since eye opening occurs at about postnatal days 13-15 it is possible that the visual information received by perirhinal cortex at this age is involved in the developmental switch in LTD mechanisms that we have observed. To examine whether the change from mGlu to muscarinic LTD arises as a consequence of visual experience, LTD was examined in dark-reared P28-35 rats. The mGlu5 antagonist MPEP prevented the induction of LTD in dark-reared P28-35 rats (2 ± 5% in MPEP and 25 ± 6% after washout of MPEP, n=5). In contrast, the inhibition of muscarinic receptors had no effect on LTD in dark-reared P28-35 rats (27 ± 5% depression, n=5).
The present results show that experience-dependent visual activity is an important factor in the modification of LTD induction mechanisms in downstream association perirhinal cortex. It is still a matter of speculation how visual experience triggers the changes in LTD mechanisms but it is likely that appropriate coordinated visual activity into perirhinal cortex somehow up-regulates muscarinic receptor expression or function whilst down-regulating mGluR expression or function.

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SA51

The role of perirhinal cortex in memory and perception: Conjunctive representations for object identification

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It is now clear that the perirhinal cortex has functions that are distinct and dissociable from those of the hippocampus, and thus recent research has focussed on the question of how best to characterise these functions. I will present a model of perirhinal cortex function that accounts for extant data, and makes novel predictions, regarding the effects of perirhinal cortex lesions on visual discrimination (Bussey & Saksida 2002). The fundamental premise of the model is that perirhinal cortex can be thought of as an extension of the hierarchically organised ventral visual stream for object identification. The perirhinal cortex is thought to reside at the top of this hierarchy, containing complex conjunctive visual representations that are important for resolving feature ambiguity in visual discriminations. I will describe novel predictions, generated by the model, which have been subsequently tested in our experiments with rhesus monkeys (Bussey et al. 2002, 2003). These computer simulations and monkey experiments reveal a perceptual-mnemonic function for perirhinal cortex, and suggest that perception and memory may not be neatly organised into anatomically distinct modules in the brain.

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SA52

Synaptic plasticity in the perirhinal cortex: A neural substrate for familiarity discrimination

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Recognition memory which requires judgements to be made about the prior occurrence of stimuli or groups of stimuli has been shown to depend upon the integrity of the perirhinal cortex. For example, electrophysiological recording experiments have revealed that changes in the responses of neurones in the perirhinal cortex are central to the ability to judge prior occurrence. Further ablation of perirhinal cortex has been shown to produce significant impairments in memory tasks requiring the ability to discriminate between novel and familiar stimuli.
Our research into the neural basis of recognition memory has focussed on exploring the hypothesis that changes in synaptic efficacy (exemplified by long term potentiation (LTP) and long term depression (LTD)) underlie the familiarity discrimination processes. To achieve this aim our strategy has been to manipulate specific receptors or signalling pathways in the perirhinal cortex and to compare the results of such manipulations across different levels of experimental analysis (cellular, systems and behavioural).
I shall present recent data from our laboratory showing that pharmacological blockade of ionotropic and metabotropic glutamate receptors in the perirhinal cortex impairs both familiarity discrimination and the induction of long-term synaptic plasticity in the perirhinal cortex. I shall also present data from experiments in which we have used viral vectors which encode a dominant negative form of CREB to block CREB-mediated transcription in the perirhinal cortex. This transduction resulted in both a behavioural impairment in object recognition memory and a failure in the maintenance of LTP in perirhinal slices. Together these results suggest that the mechanisms which underlie synaptic plasticity may provide candidate mechanisms for processes effecting recognition memory.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.