Promiscuous involvement of mGluRs in the storage of NMDAR-dependent STP

Rachael Ingram[1] & Arturas Volianskis[1,2]

Affiliations
[1]Centre for Neuroscience, Surgery and Trauma, Blizard Institute,
Barts and The London School of Medicine and Dentistry,
Queen Mary University of London, London, UK
[2]School of Biosciences, Cardiff University,
Museum Avenue, Cardiff, UK

Corresponding Author
Dr. Arturas Volianskis;
[2]School of Biosciences, Cardiff University
Museum Avenue,
Cardiff, CF10 3AX, UK
Email: VolianskisA@cardiff.ac.uk
ORCID iD: 0000-0002-2047-8269

Dr. Rachael Ingram
ORCID iD: 0000-0001-5300-4337

Keywords
Short-term potentiation (STP), Long-term potentiation (LTP), NMDA receptor (NMDAR), Metabotropic glutamate receptor (mGluR), Synaptic plasticity, Learning and memory

Summary (172 words)

Short- and long-term forms of N-methyl-D-aspartate receptor (NMDAR) dependent potentiation (most commonly termed short-term potentiation, STP and long-term potentiation, LTP) are co-induced in hippocampal slices by theta-burst stimulation (TBS), which mimics naturally occurring patterns of neuronal activity. Whilst NMDAR-dependent LTP (NMDAR-LTP) is said to be the cellular correlate of long-term memory storage, NMDAR-dependent STP (NMDAR-STP) is thought to underlie the encoding of shorter-lasting memories. The mechanisms of NMDAR-LTP have been researched much more extensively than those of NMDAR-STP, which is characterised by its extreme stimulation dependence. Thus, in the absence of low-frequency test stimulation, which is used to test the magnitude of potentiation, NMDAR-STP does not decline until the stimulation is resumed. NMDAR-STP represents, therefore, an inverse variant of Hebbian synaptic

plasticity, illustrating that inactive synapses can retain their strength unchanged until they become active again. The mechanisms, by which NMDAR-STP is stored in synapses without a decrement, are unknown and we report here that activation of metabotropic glutamate receptors (mGluRs) may be critical in maintaining the potentiated state of synaptic transmission.

Introduction

High frequency activation of excitatory glutamatergic synapses in the adult hippocampus induces two forms of *N*-methyl-d-aspartate receptor (NMDAR)-dependent long-lasting potentiation (1-4), which are most frequently referred to as short-term potentiation (STP) and long-term potentiation (LTP). NMDAR-dependent STP is often seen as the initial, transient, declining phase of potentiation that leads to a stable phase of potentiation, sustained NMDAR-dependent LTP (4). Inductions of STP and LTP differ in terms of their sensitivity to a variety of NMDAR antagonists (5-8) and second messenger inhibitors (9-12). STP and LTP also differ in terms of second messenger involvement in their expression (13-15); they are pharmacologically and physiologically distinct (16-18).

NMDAR-dependent STP is sometimes confused with NMDAR-independent types of synaptic plasticity (e.g. paired-pulse facilitation, frequency facilitation, post-tetanic potentiation) that are collectively known under the umbrella term of "short-term plasticity", sharing the acronym of STP (please see (17, 19) and also this issue of the Philosophical Transactions B (ref) for further discussion of the issues with the terminology). Due to such confusion NMDAR-dependent STP has been termed transient LTP (transient phase of LTP or t-LTP (4)) in contrast to the stable or sustained LTP (s-LTP), which is less prone to decline (20, 21). Indeed, due to its NMDAR-receptor dependence and long-lasting duration, NMDAR-dependent STP is more akin to NMDAR-dependent LTP than to the various forms of NMDAR-independent short-term plasticity. However, despite its faults, it has proven near to impossible to change the established nomenclature (22) that has been adopted and used by the field for many years. Therefore, in order to avoid any further ambiguity in the use of the acronyms, throughout the rest of this paper

we will be referring to NMDAR-dependent STP as NMDAR-STP. We will be referring to NMDAR-dependent LTP simply as LTP.

During the past 50 years LTP (1) has established itself as a reputable correlate of long-lasting memory storage (22-26) whilst the idea that NMDAR-STP could be the synaptic mechanism behind the encoding of shorter-lasting memories (27) has been slowly gaining traction over the years (4, 6, 12, 18, 28, 29). Notably, the longevity of both NMDAR-STP and LTP is not absolute but depends on the reference frame and actions of the observer, whose measurement of NMDAR-STP and LTP introduces uncertainty in the outcome of the experiments by affecting the duration of synaptic plasticity (4, 30).

NMDAR-STP and LTP are frequently co-induced and co-expressed in adult hippocampal glutamatergic synapses *in situ* (4) and *in vivo* (2). During experiments in hippocampal slice preparations potentiation of synaptic responses is probed by using low frequency presynaptic stimulation (e.g. 0.017 to 0.13 Hz), which retains the stability of baseline postsynaptic responses. After the induction of potentiation, the sensitivity of the responses to synaptic stimulation changes, and the decay of NMDAR-STP – or its depotentiation – happens readily when probed with baseline stimulation frequencies that do not affect LTP. Thus, in experiments using continuous low-frequency test stimulation NMDAR-STP decays to a stable level of LTP in about an hour (4). The depotentiation of LTP requires higher frequencies of stimulation than NMDAR-STP (e.g. 1-2 Hz, (30-33)). At baseline stimulation frequencies *in situ* LTP can last without a decrement for hours (34), whilst LTP *in vivo* has been shown to last for days (35, 36), months (37) and even years (21).

The duration of NMDAR-STP can be controlled by changing the frequency of the depotentiating stimulation (2, 4, 12, 38). NMDAR-STP declines faster in experiments with more frequent pre-synaptic stimulation than in experiments with slower stimulation (2, 4, 38). The process of the NMDAR-STP decay can be suspended by temporally pausing the afferent stimulation (4, 5, 12, 18), for a variety of time periods and up to 6 h *in situ* (4). In such experiments, the levels of NMDAR-STP are stored in synapses during periods of synaptic inactivity and NMDAR-STP does not decline until the stimulation is resumed, and synapses are

re-activated. Such use-dependent storage of potentiation can temporarily increase the dominance of some synaptic connections over others, permitting formation of dynamic cell assembles and short-term memories. Reactivation of the set cell assemblies, which have been left in a potentiated state by a momentary experience, would then be central during recall and in cognition (27). As suggested by Donald Hebb in 1961, such synaptic memory processes would be able to account for memory types that cannot be explained by either reverberatory activity or by a structural change (39). Indeed, it has been now shown that during cognition humans can hold working memory information using activity-silent synaptic mechanisms (40), relying possibly on NMDAR-STP-like processes.

At present, the mechanisms underlying the synaptic storage of NMDAR-STP are largely unknown except that it has been observed that NMDA receptors can be involved in regulating the decay of NMDAR-STP, after its initial induction (4). The involvement of metabotropic glutamate receptors (mGluRs) in the storage of NMDAR-STP has not been investigated previously and we have tested here whether or not a specific mGluR is involved in mediating the storage of NMDAR-STP.

Materials and Methods

Experiments were performed as described previously in detail (4, 5), after institutional approval and according to national and EU guidelines for animal care, using Schedule 1 procedures for tissue preparation (the UK Scientific Procedures Act, 1986). Briefly, dorsal hippocampal slices were prepared from adult Wistar rats (n = 76, 220 - 300 g, Charles River Labs UK or Envigo UK), after isoflurane anaesthesia and cervical dislocation. Field excitatory postsynaptic potentials (f-EPSPs) were recorded under submerged recording conditions, from the CA1b area of the Schaffer collaterals, which were stimulated at the border between CA3 and CA1 (0.067 Hz, detailed methods in (4)). The slopes of the f-EPSPs were measured and results are presented as Mean ± SEM (% potentiation over baseline), plotted over time at 2 min intervals (Fig 1). Potentiation was induced by theta-burst

stimulation (TBS, 4 pulses at 100 Hz repeated 10 times at a 5 Hz frequency (41, 42)) that was applied after the 30 min recording of stable baseline responses. The baseline stimulation was then stopped for 3 min to avoid contamination of the estimate of maximal NMDAR-dependent potentiation (Pmax) by post-tetanic potentiation (PTP), which is NMDAR independent (4). Following the recording of Pmax, NMDAR-STP was either allowed to decay in response to stimulation or the stimulation was discontinued for 30 min and NMDAR-STP was then seen after the resumption of the stimulation decaying to a stable level of LTP. Exponential fitting was used to determine the decay times (τ) of NMDAR-STP. The measurements of (1) Pmax, (2) the level of potentiation after the resumption of stimulation (potentiation at time zero, P_{t0}) and (3) LTP report % increase over baseline of the f-EPSP slope whilst the amplitude of NMDAR-STP (%) is the difference between the P_{t0} and LTP (4).

During the pharmacological experiments mGluR antagonists were applied after the recording of Pmax, during the 30 min delay in stimulation and kept throughout the experiment. The ligands included a variety of competitive and allosteric compounds: mGluR antagonists LY367385 (mGluR1-preferring, Group I antagonist), LY341495 (mGluR2-preferring, Group II/III antagonist) and subtype-selective allosteric inhibitors targeting mGluR1 (YM298198), mGluR5 (MTEP) and an mGluR7 inhibitor (XAP044). Compounds were purchased from either ABCAM Biochemicals UK or Tocris Bioscience UK, prepared as stock solutions according to the manufacturer's guidelines, stored frozen and added to experimental solutions when needed.

Experiments were performed in a randomized manner with control and pharmacological experiments interleaved. Paired Student's t-tests were used for the within group comparisons, unpaired t-tests were used to compare between different groups and frequency distributions were compared using F-test (GraphPad Prism). One way ANOVA followed by Bonferroni's multiple comparison test (Bonferroni's MCT) were used to compare mean values of more than two groups (GraphPad Prism).

Results

Stochastic involvement of mGluRs in the storage of NMDAR-STP

Two types of control experiments (Fig 1A, white vs black circles) were interleaved with the pharmacological experiments, which used the mGluR antagonists (Fig 1A, orange squares). In both types of the controls, TBS was applied after the 30 min recording of stable baseline responses and maximal levels of NMDAR-dependent potentiation (Pmax) were estimated after a 3-min delay to avoid NMDAR-independent PTP (4, 43). Following the recording of Pmax, NMDAR-STP was either allowed to decay in response to stimulation (white circles, Pmax = 120.7 ± 7.6 %, NMDAR-STP = 61.9 ± 5.3 %, τ = 22.8 ± 3.9 min, LTP = 45.8 ± 3.7 %, n = 15) or the stimulation was discontinued for 30 min (indicated by the thick line, black circles n = 15, Fig 1A). After the resumption of the stimulation NMDAR-STP was seen decreasing to a stable level of LTP, similar to the experiments without the delay in stimulation (black circles vs white circles, respectively Fig 1A). In the experiments with the 30 min gap in stimulation there was virtually no decrement in the levels of potentiation during the pause (Pmax - P_{t0} = 2.7 ± 4.3 %), when compared between the Pmax (109.8 ± 7.8 % over baseline, n = 15) and the level of potentiation 2 min after the resumption of stimulation (potentiation at time zero, P_{t0} = 107.1 ± 6.6 %, p = 0.53). After the resumption of the stimulation NMDAR-STP (62.4 ± 4.0 %) decayed with a time constant of 15.7 ± 2.5 min, to a 44.7 ± 4.8 % level of LTP above baseline (black circles, Fig 1A).

Pharmacological experiments (orange squares Fig 1A, n = 75) using the mGluR antagonists were performed in the same way as the control experiments with the 30 min pause in stimulation (black circles Fig 1A) except that ligands were bath applied after the recording of the Pmax, and then kept throughout (dashed bar, Fig 1A). In these experiments there was a significant decrement (20.5 ± 4.0 %) in the levels of potentiation when compared between the Pmax and P_{t0} (111.5 ± 3.6 vs 91.1 ± 3.6 % respectively, $p < 0.00001$). NMDAR-STP (46.1 ± 3.3 %) declined faster than in the control (τ = 8.4 ± 0.8 vs 15.7 ± 2.5 min respectively, p = 0.0007) to the same level of LTP as in the control (45.0 ± 1.6 vs 44.7 ± 4.8 % respectively, p = 0.95).

Examination of the pharmacological dataset revealed a large number of single experiments that were virtually NMDAR-STP lacking (see the waveforms in

Fig 1). Concurrently, many of the other experiments seemed to be similar to the controls and we sought a strategy to sort the results in an unbiased way. As reported above, the amplitude of NMDAR-STP in the control experiments with the 30 min gap in stimulation was 62.4 % and σ was 15.4 %. All the single experiments, in both the control and the pharmacological datasets, were therefore sorted according to the magnitude of NMDAR-STP after the resumption of the stimulation by using the empirical 95% rule for separating independent Gaussian distributions (i.e. 62.4 % - 2*σ; NMDAR-STP < 31.5 % and NMDAR-STP > 31.5 %). Notably, the control dataset did not contain any experiments in which NMDAR-STP was smaller than 31.5 % (range 39.9 to 93.2 %, black bars, Fig 1B) whilst 28 out of 75 pharmacological experiments showed NMDAR-STPs that were smaller than 31.5 % (range -29.2 to 30.4 %, yellow bars, Fig 1B). The residual 47 experiments, in which NMDAR-STP was larger than 31.5 %, are shown plotted in red (range 32.4 to112.3 %, Fig 1B). The datasets were fitted with Gaussian distributions demonstrating that the "large" NMDAR-STP group (NMDAR-STP > 31.5%, red bars, Fig 1B) was virtually indistinguishable from the control group in terms of its mean NMDAR-STP values and σ ($F(2, 12) = 0.0492$, $p = 0.9522$). The mean NMDAR-STP values and σ in the "small" NMDAR-STP group (NMDAR-STP < 31.5 %) were statistically different from those in both the "large" NMDAR-STP group and the control experiments ($F(4, 36) = 49.38$, $p < 0.0001$).

The datasets that emerged through the sorting procedure revealed that the mean Pmax values were very similar when compared between the "small" and "large" NMDAR-STP groups (110.3 ± 5.9 vs 112.3 ± 4.7 % respectively, $p = 0.79$, yellow vs red squares, Fig 1C). The levels of LTP were also similar between the two datasets (44.7 ± 2.3 vs 45.2 ± 2.3 %, $p = 0.88$). However, storage of NMDAR-STP was clearly disrupted in the "small" NMDAR-STP group that showed a 50 % decrement in potentiation when compared between the Pmax and the P_{t0} (Pmax - P_{t0} = 49.9 ± 5.5 %, $p < 0.0001$). The storage of NMDAR-STP was unaffected in the "large" NMDAR-STP group (Pmax - P_{t0} = 2.9 ± 3.7 %, $p = 0.43$). The amount of decrement in potentiation during the 30 min period without stimulation differed significantly when compared between the control and the "small" NMDAR-STP group (Fig 1D1 & D2, $p < 0.0001$ Bonferroni's MCT, black vs yellow), whilst there was no difference when compared between the control and the large NMDAR-STP

group (Fig 1D1 & D2, p > 0.99 Bonferroni's MCT, black vs red). We were interested whether the observations of stored and disrupted NMDAR-STP were stemming from particular animals or could be attributed to the application of mGluR antagonists. Experiments in slices from the same animals showed incidences of both stored and disrupted NMDAR-STP (Table 1) and we concluded that application of mGluR antagonists can disrupt NMDAR-STP in an unpredictable fashion.

Involvement of multiple mGluRs in the storage of NMDAR-STP

As described above, a significant proportion of the pharmacological experiments (37 %) showed an almost complete disappearance of NMDAR-STP when mGluR receptor inhibitors were applied during the pause in stimulation. Surprisingly, disrupted NMDAR-STP experiments were seen with both Group I (41 % n = 51, Fig 2A) and Group II/III mGluR inhibitors (29 % n = 24, Fig 2B). In all of these experiments the levels of Pmax and LTP remained consistent with those in the controls, suggesting an NMDAR-STP specific effect of the antagonists.

Indeed, all the antagonists that were used in this study could produce a loss of NMDAR-STP, irrespective of their subtype preference, whilst maintenance of LTP was never affected by the compounds (Fig 3).

In terms of the Group I mGluRs, NMDAR-STP was disrupted in 36 % of experiments that used 1 μM mGluR1-selective antagonist YM298198 (n = 11, Fig 3A). Inhibition of mGluR5 receptors whilst using the mGluR5-selective antagonist MTEP (1 μM) could also produce disruption of NMDAR-STP (30 % of total experiments, n = 10, Fig 3B). Co-inhibition of mGluR1 and mGluR5 by co-application of YM298198 and MTEP disrupted NMDAR-STP in 45 % of the experiments (Fig 3C, n = 11). Similarly, the storage of NMDAR-STP was disrupted in 47 % of total experiments (n = 19) when using the mGluR1-preferring concentration of LY367385 (30 μM, Fig 3D).

Baseline synaptic transmission was not affected by any of the mGluR antagonists that were used in the experiments. The level of synaptic transmission was 3.26 ± 0.68 % (not shown) of baseline after 30 min application of the combination of 1 μM YM298198 and 1 μM MTEP. Likewise, the transmission was

0.94 ± 0.74 % (not shown) of baseline after 30 min application of 30 μM LY367385. Notably, in experiments without the gap in stimulation, 30 μM LY367385 did not inhibit the induction of NMDAR-STP and did not disrupt its decay (Fig 3D, grey squares). Here, the antagonist was applied prior to the TBS, and kept throughout. 30 μM LY367385 had no effect on baseline synaptic transmission after the gap in stimulation in experiments without the TBS, suggesting that the disruptive effect of LY367385 is specific to the storage of NMDAR-STP (Fig 3D, green squares).

Similarly to the Group I experiments, application of the Group II/III mGluR inhibitors could also disrupt NMDAR-STP. Thus, NMDAR-STP was disrupted in 44 % of cases (n = 9, Fig 3E) using group II/III-preferring concentration of the competitive mGluR antagonist LY341495 (1 μM). NMDAR-STP was also disrupted in 33 % of experiments using the mGluR7-selective antagonist XAP044 (1 μM, n = 9, Fig 3F). Neither LY341495 nor XAP044 affect baseline synaptic transmission (44, 45).

Notably, we have never observed any effects on NMDAR-STP in experiments that used a lower, more mGluR2/mGluR3-preferring concentration of LY341495 (0.1 μM, n = 6, not shown). In these experiments there was no significant decrement from Pmax to P_{t0} (3.8 ± 11.7 %, p = 0.76) and NMDAR-STP (63.5 ± 12.1 %) declined with τ 13.8 ± 2.5 min to an LTP level of 46.8 ± 8.9 % (Pmax = 114.0 ± 14.5 %, P_{t0} = 110.2 ± 12.7 %). Adjusting the overall experimental numbers for the lack of effects of the low concentration of LY341495 suggests that inhibition of both Group I (21/51) and Group II/III (7/18) mGluRs can disrupt storage of NMDAR-STP with a similar likelihood of 41 % vs 39 % of cases, respectively.

Discussion

It is well known that mGluRs can have a role in the induction of synaptic plasticity (reviewed in (46, 47)), whereas their involvement in maintaining the potentiated state of synaptic transmission is an unexpected observation. Data, which are presented in this paper, suggest that inhibition of Group I mGluRs, and also Group II/III mGluRs, can prevent synapses from storing NMDAR-STP during

periods without synchronous synaptic activation. We do not know whether storage of NMDAR-STP requires constitutive activation of mGluRs (48-50) or whether they are activated by ambient glutamate, or glutamate that is released during spontaneous synaptic events. Our data suggest, however, that the process of NMDAR-STP storage is not passive but metabolically active in that application of mGluR antagonist can disrupt this process.

It is surprising to observe that mGluRs, activating completely different second messenger systems, are involved in maintaining the synaptic strength during the storage of NMDAR-STP. Involvement of Group II mGluRs will have to be confirmed by using compounds that are more mGluR2/3 selective than LY341495, which did not disrupt NMDAR-STP at a low concentration. On the other hand, the involvement of Group I and Group III receptors in the storage of NMDAR-STP is highly likely. Group I and Group III mGluRs involve different effector molecules (Gq and Gi, respectively) and have opposing effects on adenylyl cyclase activity (51). The nature of such convergent involvement of different mGluRs in the storage of NMDAR-STP requires further research. Notably, reduced induction of NMDAR-STP, and working memory deficits, have been reported in the mGluR7 knockout mice whilst LTP was unaffected, linking involvement of Group III mGluRs to both NMDAR-STP and working memory (52-54). On the other hand, a relationship between Group I mGluRs, synaptic plasticity and memory has also been suggested (e.g. (55), for review see (46, 47)). Interestingly, activation of Group I mGluRs can potentiate NMDAR responses (56, 57) whilst activation of NMDARs can regulate the decay of NMDAR-STP after its induction (4). Such interaction between Group I mGluRs and NMDARs, which might be strengthened by the induction of NMDAR-STP, will need to be researched in future studies.

As described above, NMDAR-STP was disrupted in only about 30 to 50 % of our pharmacological experiments, dependent on the antagonist used. Such disruption of NMDAR-STP has not been observed in the control experiments of the current study and was not dependent on the animal identity. Reliable storage of STP has also been reported in the previous publications, which investigated storage of potentiation during time periods without stimulation (4, 5, 12, 18). The reason for the inconsistency of the effects of mGluR antagonists is unknown and

will need to be investigated further. Notably, it has been previously shown that 3-isobutyl-1-methylxanthine (IBMX), a phosphodiesterase inhibitor that raises intracellular cAMP, can occlude induction of NMDAR-STP in about 25 % of cases (16). It is, therefore, possible that several parallel second messenger cascades, competing for some common mechanism of expression of plasticity, are involved in NMDAR-STP storage, which might explain its sensitivity to inhibition by ligands that target different mGluR groups. We cannot exclude, however, the possibility that mGluRs that are mediating the storage of NMDAR-STP are composed of unconventional subunit combinations (58).

NMDAR-STP is only sometimes (4) but not always (5, 28, 38) induced as a uniformly decaying exponential phenomenon and has been subdivided into fast- and slow-decaying exponential components termed NMDAR-STP1 and NMDAR-STP2, respectively (5). Induction of NMDAR-STP1 and NMDAR-STP2 relies on activation of NMDARs composed of different subunits (5, 6, 8) and it is therefore possible that different types of NMDAR-STP involve activation of different types of mGluRs, a question that we could not address in the current study. To answer this question, NMDAR-STP1 and NMDAR-STP2 would need to be induced selectively, re-examining the effects of mGluR antagonists on the processes of their storage and decay.

In conclusion, and to the best of our knowledge, this is the first report that documents the involvement of glutamate receptors in the storage of NMDAR-STP and shows that inhibition of mGluRs (Groups I & III, particularly) can lead to selective disruption of NMDAR-STP without affecting LTP. It supports previous evidence that NMDAR-STP is a distinct form of synaptic plasticity (2, 4, 5, 12, 16, 17, 38), having unique pharmacological profile (5, 6) and computational capacity (18, 59, 60) whereas its putative physiological role in short-term memory processes, which was originally suggested by G.V. Goddard in the 1980s celebration of D.O. Hebb (27), is yet to be confirmed *in vivo*.

Acknowledgements
We would like to acknowledge The Royal Society (RSG\R1\180384; AV) and the Blizard Institute, QMUL for supporting this study.

List of Abbreviations

NMDAR-STP	Short-term potentiation
LTP	Long-term potentiation
PTP	Post-tetanic potentiation
NMDAR	N-methyl-D-aspartate receptor
mGluR	Metabotropic glutamate receptor
fEPSP	Field excitatory postsynaptic potential
IBMX	3-isobutyl-1-methylxanthine
SEM	Standard error of the mean
TBS	Theta-burst stimulation
cAMP	Cyclic adenosine monophosphate
NAM	Negative allosteric modulator

Supplementary material

None included.

Authors' contributions

RI and AV designed the study. RI performed the experiments. RI and AV analysed the data. RI wrote the first draft of the manuscript and both RI and AV finalised and approved the paper.

Funding

Supported by The Royal Society (RSG\R1\180384) and the Blizard Institute.

Availability of data and materials

All data and their analyses are included in this paper any additional information is available from the corresponding author on reasonable request.

Ethics

Experiments were performed after institutional approval and according to national and EU guidelines for animal care using Schedule 1 procedures for tissue preparation (the UK Scientific Procedures Act, 1986).

Consent for publication

Not applicable.

Competing interests

The authors have no competing interests to declare.

Figure captions

Fig. 1 Involvement of mGluRs in the storage of NMDAR-STP.

(A) Theta-burst stimulation (TBS, timing indicated by the arrow) was used to induce NMDAR-STP and LTP of f-EPSPs after recording of stable baseline responses (Baseline; black waveforms) in three groups of experiments (experiment numbers are displayed on the panel). In the first group of control experiments (white circles; no gap control) NMDAR-STP was depotentiated by stimulation (1/15 s, 0.067 Hz) immediately after the recording of the maximal levels of NMDAR-dependent potentiation (Pmax; red waveform) to a stable level of LTP (blue waveform). In the second group of control experiments (black circles, 30 min gap control) NMDAR-STP was stored for 30 min by a delay (gap) in stimulation (indicated by the thick black line; No stim). After the resumption of the stimulation (P_{t0}; green waveform) NMDAR-STP was depotentiated to a stable level of LTP (blue waveform). In the third group of experiments (orange squares; mGluR exps) an mGluR inhibitor was applied after the recording of Pmax, during the 30 min gap in stimulation and throughout the rest of the experiments (indicated by the dashed line). In this group of experiments NMDAR-STP could be either stored as in controls (outcome "1", green f-EPSP (P_{t0}) overlapping the red, Pmax) or not stored (outcome "2", green f-EPSP (P_{t0}) overlapping the blue, LTP).

(B) Frequency distributions of NMDAR-STP amplitudes (10 % bins) after the resumption of the stimulation for controls (black) and for experiments using mGluR inhibitors in which NMDAR-STP was either disrupted (yellow, NMDAR-STP < 31.5 %) or stored successfully (red; NMDAR-STP >31.5 %) were fitted with Gaussian curves (dashed lines). When comparing the Gaussian curves, the mean amplitude and σ of the disrupted NMDAR-STP distribution (yellow, 19.9 ± 8.0 %) were significantly smaller than those in control distribution (black, 60.1 ± 16.8 %, $p < 0.0001$), or in experiments with successful storage (red distribution, 61.6 ± 18.8 %, $p < 0.0001$).

(C) The datasets from B are shown plotted as mean values of potentiation ± SEM, over time. After the resumption of stimulation, the mean NMDAR-STP amplitude in the stored NMDAR-STP group (red squares) was 64.2 ± 2.6 % whilst NMDAR-STP in the disrupted group (15.8 ± 2.4) was much smaller. The values of Pmax and LTP were consistent with those in the control (black circles). A large decrement in potentiation during the 30 min gap in stimulation (Pmax vs P_{t0}) was seen in the disrupted NMDAR-STP group but not in the stored.

(D1) Decrement in NMDAR-STP during the 30 min gap in stimulation, individual experiment data from the datasets in B (Pmax - P_{t0}; mean values ± confidence intervals are also displayed).

(D2) The mean decrement in NMDAR-STP ± SEM (data from D1) during the gap in stimulation: the control (C, black), the stored mGluR group (S, red) and the disrupted NMDAR-STP group (D, yellow). Ordinary one-way ANOVA $F(2,87) = 33.09$; $P < 0.001$, significant differences after Bonferroni's MCT.

Fig. 2 Group I and Group II/III inhibitors cause disruption of NMDAR-STP.

(A) Application of group I mGluR antagonists during the gap in stimulation disrupted storage of NMDAR-STP in 21 out of 51 experiments (42.2 %). The stored NMDAR-STP group (Pmax - P_{t0} = 4.0 ± 4.8 %, p = 0.4): Pmax = 116.1 ± 6.0 %, P_{t0} = 112.0 ± 4.2 %, NMDAR-STP = 65.3 ± 3.1 %, τ = 9.3 ± 1.0 min, LTP = 46.8 ± 2.9 %. The disrupted NMDAR-STP group (Pmax - P_{t0} = 50.5 ± 6.8 %, p < 0.00001): Pmax = 112.8 ± 6.4 %, P_{t0} = 62.4 ± 3.4 %, NMDAR-STP = 15.9 ± 3.1 %, LTP = 46.4 ± 2.6 %. We are not reporting τ values of the disrupted NMDAR-STP groups due to ambiguity of measurements in this and subsequent figures.

(B) Application of group II/III mGluR antagonists also disrupted the storage of NMDAR-STP (7/24 29.1 %). The stored NMDAR-STP group (Pmax - P_{t0} = 1.0 ± 5.8 %, p = 0.87): Pmax = 105.6 ± 7.4 %, P_{t0} = 104.6 ± 5.5 %, NMDAR-STP = 62.3 ± 4.6 %, τ = 10.2 ± 1.3 min, LTP = 42.3 ± 3.6 %. The disrupted NMDAR-STP group (Pmax - P_{t0} = 48.2 ± 9.8 %, p < 0.003): Pmax = 102.8 ± 14.4 %, P_{t0} = 54.7 ± 7.1 %, NMDAR-STP = 15.3 ± 3.4 %, LTP = 39.4 ± 4.6 %.

Fig. 3 No receptor specificity for the mGluR mediated disruption of NMDAR-STP

(A) Application of the mGluR1 antagonist YM298198 disrupted storage of NMDAR-STP in 4 out of 11 experiments (36.4 %). The stored NMDAR-STP group (Pmax - P_{t0} = 11.2 ± 5.7 %, p = 0.1): Pmax = 131.4 ± 11.3 %, P_{t0} = 120.2 ± 8.3 %, NMDAR-STP = 64.9 ± 8.7 %, τ = 13.5 ± 1.9 min, LTP = 55.3 ± 4.1 %. The disrupted NMDAR-STP group (Pmax - P_{t0} = 52.6 ± 7.9 %, p = 0.007): Pmax = 132.7 ± 9.2 %, P_{t0} = 80.0 ± 7.5 %, NMDAR-STP = 22.1 ± 2.6 %, LTP = 57.9 ± 7.7 %.

(B) mGluR5 antagonist MTEP disrupted storage of NMDAR-STP in 3 out of 10 experiments (30 %). The stored NMDAR-STP group (Pmax - P_{t0} = -6.8 ± 9.6 %, p = 0.5): Pmax = 100.4 ± 8.3 %, P_{t0} = 107.2 ± 9.6 %, NMDAR-STP = 63.0 ± 7.4 %, τ = 6.7 ± 1.3 min, LTP = 44.2 ± 5.9 %. The disrupted NMDAR-STP group (Pmax - P_{t0} = 43.6 ± 9.8 %, p = 0.047): Pmax = 110.8 ± 9.3 %, P_{t0} = 67.2 ± 2.8 %, NMDAR-STP = 23.9 ± 0.7 %, LTP = 43.3 ± 3.4 %.

(C) Co-application of YM298198 (YM) and MTEP disrupted storage of NMDAR-STP 45% of experiments (5/11). The stored NMDAR-STP group (Pmax - P_{t0} = -6.0 ± 12.8 %, p = 0.7): Pmax = 107.0 ± 14.7 %, P_{t0} = 113.0 ± 8.0 %, NMDAR-STP = 66.9 ± 4.8 %, τ = 11.2 ± 2.8 min, LTP = 46.2 ± 8.1 %. The disrupted NMDAR-STP group (Pmax - P_{t0} = 38.1 ± 9.9 %, p = 0.02): Pmax = 91.4 ± 10.8 %, P_{t0} = 53.3 ± 6.3 %, NMDAR-STP = 12.4 ± 6.6 %, LTP = 41.0 ± 4.6 %.

(D) Application of mGluR1 preferring antagonist LY367385 disrupted storage of NMDAR-STP in 9/19 experiments (47 %). The stored NMDAR-STP group (Pmax - P_{t0} = 12.6 ± 8.9 %, p = 0.2): Pmax = 121.8 ± 11.6 %, P_{t0} = 109.1 ± 7.9 %, NMDAR-STP = 66.1 ± 4.8 %, τ = 6.9 ± 1.5 min, LTP = 43.0 ± 5.2 %. The disrupted NMDAR-STP group (Pmax - P_{t0} = 58.6 ± 14.1 %, p = 0.003): Pmax = 116.5 ± 11.4 %, P_{t0} = 57.9 ± 4.6 %, NMDAR-STP = 12.5 ± 5.9 %, LTP = 45.4 ± 3.3 %. In experiments without a gap, LY367385 did not affect induction of plasticity and did not disrupt decay of NMDAR-STP, when applied prior to TBS (grey dashed bar) and kept throughout (grey squares); Pmax 93.9 ± 11.3 %, NMDAR-STP 51.2 ± 5.8 %, τ = 11.0 ± 1.1 min, LTP 37.8 ± 3.7 %. In gap experiments without TBS, LY367385 did not disrupt baseline transmission (green squares, Pmax - P_{t0} = 1.4 ± 3.7 %, p = 0.7).

(E) Application of 1 µM Group II (mGluR 2 & 3) and Group III (mGluRs 4, 6, 7 & 8) antagonist LY341495 disrupted storage of NMDAR-STP in 4/9 experiments (44 %). The stored NMDAR-STP group (Pmax - P_{t0} = 0.6 ± 11.2 %, p = 0.96): Pmax = 106.0 ± 14.1 %, P_{t0} = 105.4 ± 6.1 %, NMDAR-STP = 65.7 ± 5.2 %, τ = 9.3 ± 2.1 min, LTP = 39.7 ± 4.9 %. The disrupted NMDAR-STP group (Pmax - P_{t0} = 60.3 ± 8.2 %, p = 0.005): Pmax = 113.2 ± 6.3 %, P_{t0} = 52.9 ± 4.1 %, NMDAR-STP = 12.9 ± 3.9 %, LTP = 40.0 ± 1.3 %.

(F) The mGluR7 antagonist XAP044 disrupted storage of NMDAR-STP in 3 out of 9 experiments (33 %). The stored NMDAR-STP group (Pmax - P_{t0} = -1.4 ± 9.0 %, p = 0.88): Pmax = 96.9 ± 10.9 %, P_{t0} = 98.4 ± 8.4 %, NMDAR-STP = 58.3 ± 4.8 %, τ = 7.5 ± 1.0 min, LTP = 40.1 ± 4.1 %. The disrupted NMDAR-STP group (Pmax - P_{t0} = 31.9 ± 17.4 %, p = 0.2): Pmax = 88.9 ± 34.7 %, P_{t0} = 57.0 ± 17.6 %, NMDAR-STP = 18.5 ± 6.3 %, LTP = 38.5 ± 11.9 %.

References

1. Bliss TV, Lomo T. Long–lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. J Physiol. 1973;232(2):331–56.
2. McNaughton BL. Long–term synaptic enhancement and short–term potentiation in rat fascia dentata act through different mechanisms. J Physiol. 1982;324:249–62.
3. Collingridge GL, Kehl SJ, McLennan H. Excitatory amino acids in synaptic transmission in the Schaffer collateral–commissural pathway of the rat hippocampus. J Physiol. 1983;334:33–46.
4. Volianskis A, Jensen MS. Transient and sustained types of long–term potentiation in the CA1 area of the rat hippocampus. J Physiol. 2003;550(Pt 2):459–92.
5. Volianskis A, Bannister N, Collett VJ, Irvine MW, Monaghan DT, Fitzjohn SM, et al. Different NMDA receptor subtypes mediate induction of long–term potentiation and two forms of short–term potentiation at CA1 synapses in rat hippocampus in vitro. J Physiol. 2013;591(4):955–72.
6. Ingram R, Kang H, Lightman S, Jane DE, Bortolotto ZA, Collingridge GL, et al. Some distorted thoughts about ketamine as a psychedelic and a novel hypothesis based on NMDA receptor–mediated synaptic plasticity. Neuropharmacology. 2018;142:30–40.
7. Malenka RC. Postsynaptic factors control the duration of synaptic enhancement in area CA1 of the hippocampus. Neuron. 1991;6(1):53–60.
8. Ingram R, Volianskis A. L689,560 is a potent inhibitor of short-term potentiation in the hippocampus. Br J Pharmacol. 2019;176(16):3068–9.
9. Malinow R, Madison DV, Tsien RW. Persistent protein kinase activity underlying long–term potentiation. Nature. 1988;335(6193):820–4.
10. Bortolotto ZA, Collingridge GL. Involvement of calcium/calmodulin–dependent protein kinases in the setting of a molecular switch involved in hippocampal LTP. Neuropharmacology. 1998;37(4–5):535–44.
11. Toni N, Buchs PA, Nikonenko I, Bron CR, Muller D. LTP promotes formation of multiple spine synapses between a single axon terminal and a dendrite. Nature. 1999;402(6760):421–5.
12. Pradier B, Lanning K, Taljan KT, Feuille CJ, Nagy MA, Kauer JA. Persistent but Labile Synaptic Plasticity at Excitatory Synapses. J Neurosci. 2018;38(25):5750–8.
13. Abel T, Nguyen PV, Barad M, Deuel TA, Kandel ER, Bourtchouladze R. Genetic demonstration of a role for PKA in the late phase of LTP and in hippocampus–based long–term memory. Cell. 1997;88(5):615–26.
14. Ho N, Liauw JA, Blaeser F, Wei F, Hanissian S, Muglia LM, et al. Impaired synaptic plasticity and cAMP response element–binding protein activation in Ca2+/calmodulin–dependent protein kinase type IV/Gr–deficient mice. J Neurosci. 2000;20(17):6459–72.
15. Osten P, Valsamis L, Harris A, Sacktor TC. Protein synthesis–dependent formation of protein kinase Mzeta in long–term potentiation. J Neurosci. 1996;16(8):2444–51.

16. Schulz PE, Fitzgibbons JC. Differing mechanisms of expression for short-term and long-term potentiation. J Neurophysiol. 1997;78(1):321-34.
17. Volianskis A, France G, Jensen MS, Bortolotto ZA, Jane DE, Collingridge GL. Long-term potentiation and the role of N-methyl-D-aspartate receptors. Brain Res. 2015;1621:5-16.
18. Volianskis A, Collingridge GL, Jensen MS. The roles of STP and LTP in synaptic encoding. PeerJ. 2013;1:e3.
19. France G, Volianskis R, Ingram R, Bannister N, Rotharmel R, Irvine MW, et al. Differential regulation of STP, LTP and LTD by structurally diverse NMDA receptor subunit-specific positive allosteric modulators. Neuropharmacology. 2022;202:108840.
20. Raymond CR. LTP forms 1, 2 and 3: different mechanisms for the "long" in long-term potentiation. Trends Neurosci. 2007;30(4):167-75.
21. Abraham WC. How long will long-term potentiation last? Philos Trans R Soc Lond B Biol Sci. 2003;358(1432):735-44.
22. Bliss TV, Collingridge GL. A synaptic model of memory: long-term potentiation in the hippocampus. Nature. 1993;361(6407):31-9.
23. Stevens CF. A million dollar question: does LTP = memory? Neuron. 1998;20(1):1-2.
24. Martin SJ, Grimwood PD, Morris RG. Synaptic plasticity and memory: an evaluation of the hypothesis. Annu Rev Neurosci. 2000;23:649-711.
25. Nabavi S, Fox R, Proulx CD, Lin JY, Tsien RY, Malinow R. Engineering a memory with LTD and LTP. Nature. 2014;511(7509):348-52.
26. Aggleton JP, Morris RGM. Memory: Looking back and looking forward. Brain Neurosci Adv. 2018;2:2398212818794830.
27. Goddard GV. Component Properties of the Memory Machine: Hebb Revisited. In: Jusczyk PW, Klein RM, editors. The Nature of Thought: Essays in Honor of Do Hebb. eBook (first edition 1981) ed. New Jersey.: Psychology Press.; 2014. p. 231-47.
28. Erickson MA, Maramara LA, Lisman J. A single brief burst induces GluR1-dependent associative short-term potentiation: a potential mechanism for short-term memory. J Cogn Neurosci. 2010;22(11):2530-40.
29. Lansner A, Fiebig F, Herman P. Fast Hebbian plasticity and working memory. Curr Opin Neurobiol. 2023;83:102809.
30. Barrionuevo G, Schottler F, Lynch G. The effects of repetitive low frequency stimulation on control and "potentiated" synaptic responses in the hippocampus. Life Sci. 1980;27(24):2385-91.
31. Staubli U, Lynch G. Stable depression of potentiated synaptic responses in the hippocampus with 1-5 Hz stimulation. Brain Res. 1990;513(1):113-8.
32. Fujii S, Saito K, Miyakawa H, Ito K, Kato H. Reversal of long-term potentiation (depotentiation) induced by tetanus stimulation of the input to CA1 neurons of guinea pig hippocampal slices. Brain Res. 1991;555(1):112-22.
33. Bashir ZI, Collingridge GL. An investigation of depotentiation of long-term potentiation in the CA1 region of the hippocampus. Exp Brain Res. 1994;100(3):437-43.

34. Andersen P, Sundberg SH, Sveen O, Wigstrom H. Specific long-lasting potentiation of synaptic transmission in hippocampal slices. Nature. 1977;266(5604):736-7.
35. Bliss TV, Gardner-Medwin AR. Long-lasting potentiation of synaptic transmission in the dentate area of the unanaestetized rabbit following stimulation of the perforant path. J Physiol. 1973;232(2):357-74.
36. Racine RJ, Milgram NW, Hafner S. Long-term potentiation phenomena in the rat limbic forebrain. Brain Res. 1983;260(2):217-31.
37. Douglas RM, Goddard GV. Long-term potentiation of the perforant path-granule cell synapse in the rat hippocampus. Brain Res. 1975;86(2):205-15.
38. Racine RJ, Milgram NW. Short-term potentiation phenomena in the rat limbic forebrain. Brain Res. 1983;260(2):201-16.
39. Hebb DO. Distinctive features of learning in the higher animal. In: Delafresnaye JF, editor. Brain mechanisms and learning, a Symposium. Oxford: Blackwell; 1961. p. 37-46.
40. Rose NS, LaRocque JJ, Riggall AC, Gosseries O, Starrett MJ, Meyering EE, et al. Reactivation of latent working memories with transcranial magnetic stimulation. Science. 2016;354(6316):1136-9.
41. Larson J, Wong D, Lynch G. Patterned stimulation at the theta frequency is optimal for the induction of hippocampal long-term potentiation. Brain Res. 1986;368(2):347-50.
42. Larson J, Lynch G. Induction of synaptic potentiation in hippocampus by patterned stimulation involves two events. Science. 1986;232(4753):985-8.
43. Stevens CF, Tonegawa S, Wang Y. The role of calcium-calmodulin kinase II in three forms of synaptic plasticity. Curr Biol. 1994;4(8):687-93.
44. Fitzjohn SM, Bortolotto ZA, Palmer MJ, Doherty AJ, Ornstein PL, Schoepp DD, et al. The potent mGlu receptor antagonist LY341495 identifies roles for both cloned and novel mGlu receptors in hippocampal synaptic plasticity. Neuropharmacology. 1998;37(12):1445-58.
45. Gee CE, Peterlik D, Neuhauser C, Bouhelal R, Kaupmann K, Laue G, et al. Blocking metabotropic glutamate receptor subtype 7 (mGlu7) via the Venus flytrap domain (VFTD) inhibits amygdala plasticity, stress, and anxiety-related behavior. J Biol Chem. 2014;289(16):10975-87.
46. Anwyl R. Metabotropic glutamate receptor-dependent long-term potentiation. Neuropharmacology. 2009;56(4):735-40.
47. Collingridge GL, Abraham WC. Glutamate receptors and synaptic plasticity: The impact of Evans and Watkins. Neuropharmacology. 2022;206:108922.
48. Kammermeier PJ. Constitutive activity of metabotropic glutamate receptor 7. BMC Neurosci. 2015;16:17.
49. Panaccione I, King R, Molinaro G, Riozzi B, Battaglia G, Nicoletti F, et al. Constitutively active group I mGlu receptors and PKMzeta regulate synaptic transmission in developing perirhinal cortex. Neuropharmacology. 2013;66:143-50.

50. Ango F, Prezeau L, Muller T, Tu JC, Xiao B, Worley PF, et al. Agonist–independent activation of metabotropic glutamate receptors by the intracellular protein Homer. Nature. 2001;411(6840):962–5.
51. Niswender CM, Conn PJ. Metabotropic glutamate receptors: physiology, pharmacology, and disease. Annu Rev Pharmacol Toxicol. 2010;50:295–322.
52. Bushell TJ, Sansig G, Collett VJ, van der Putten H, Collingridge GL. Altered short–term synaptic plasticity in mice lacking the metabotropic glutamate receptor mGlu7. ScientificWorldJournal. 2002;2:730–7.
53. Holscher C, Schmid S, Pilz PK, Sansig G, van der Putten H, Plappert CF. Lack of the metabotropic glutamate receptor subtype 7 selectively impairs short–term working memory but not long–term memory. Behav Brain Res. 2004;154(2):473–81.
54. Holscher C, Schmid S, Pilz PK, Sansig G, van der Putten H, Plappert CF. Lack of the metabotropic glutamate receptor subtype 7 selectively modulates Theta rhythm and working memory. Learn Mem. 2005;12(5):450–5.
55. Balschun D, Manahan–Vaughan D, Wagner T, Behnisch T, Reymann KG, Wetzel W. A specific role for group I mGluRs in hippocampal LTP and hippocampus–dependent spatial learning. Learn Mem. 1999;6(2):138–52.
56. Doherty AJ, Palmer MJ, Bortolotto ZA, Hargreaves A, Kingston AE, Ornstein PL, et al. A novel, competitive mGlu(5) receptor antagonist (LY344545) blocks DHPG–induced potentiation of NMDA responses but not the induction of LTP in rat hippocampal slices. Br J Pharmacol. 2000;131(2):239–44.
57. Tigaret CM, Olivo V, Sadowski J, Ashby MC, Mellor JR. Coordinated activation of distinct Ca(2+) sources and metabotropic glutamate receptors encodes Hebbian synaptic plasticity. Nat Commun. 2016;7:10289.
58. McCullock TW, Kammermeier PJ. The evidence for and consequences of metabotropic glutamate receptor heterodimerization. Neuropharmacology. 2021;199:108801.
59. Fiebig F, Herman P, Lansner A. An Indexing Theory for Working Memory Based on Fast Hebbian Plasticity. eNeuro. 2020;7(2).
60. Fiebig F, Lansner A. A Spiking Working Memory Model Based on Hebbian Short–Term Potentiation. J Neurosci. 2017;37(1):83–96.

Fig. 1 Promiscuous mGluRs; Ingram R & Volianskis A 2024

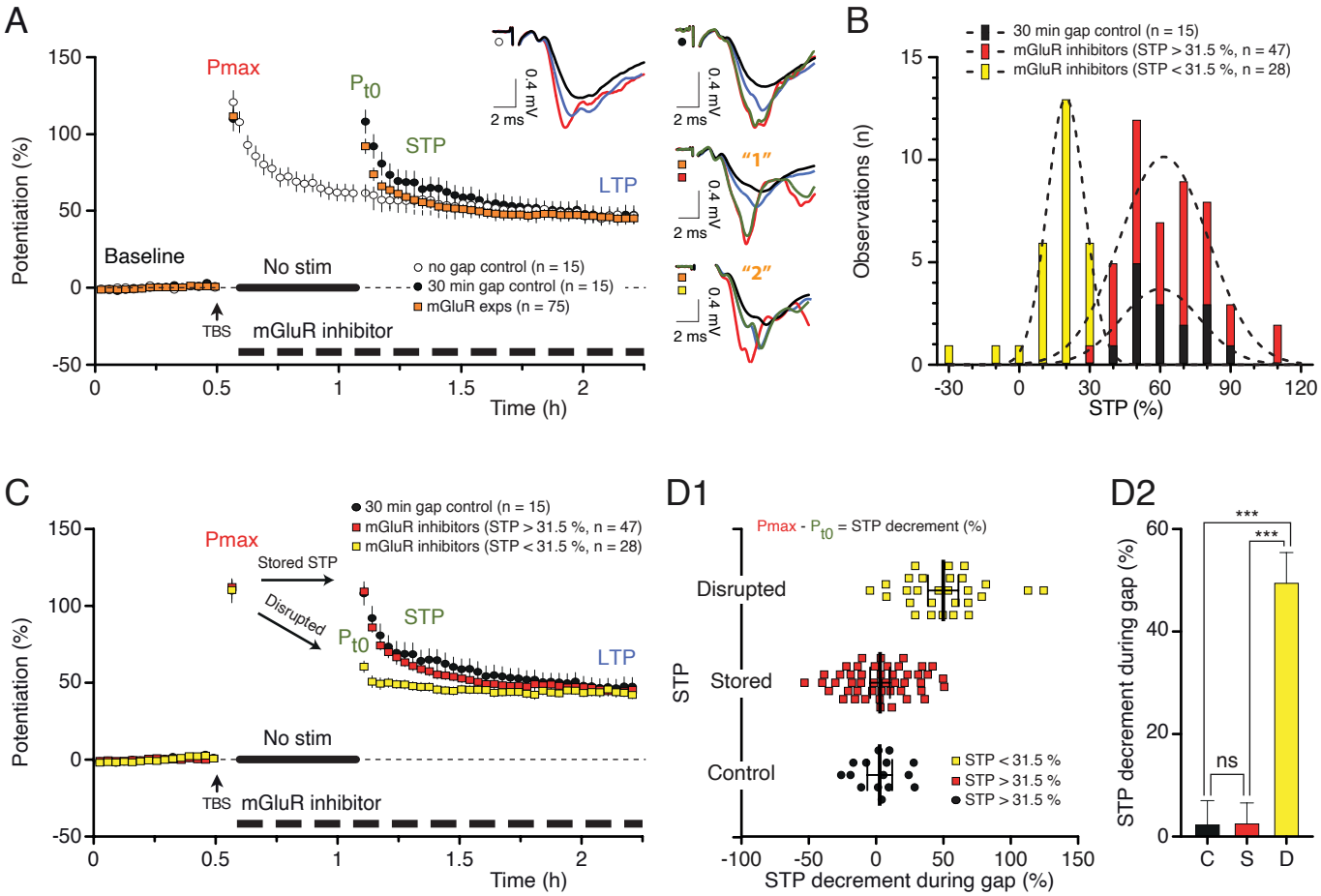

Fig.2 Promiscuous mGluRs; Ingram R & Volianskis A 2024

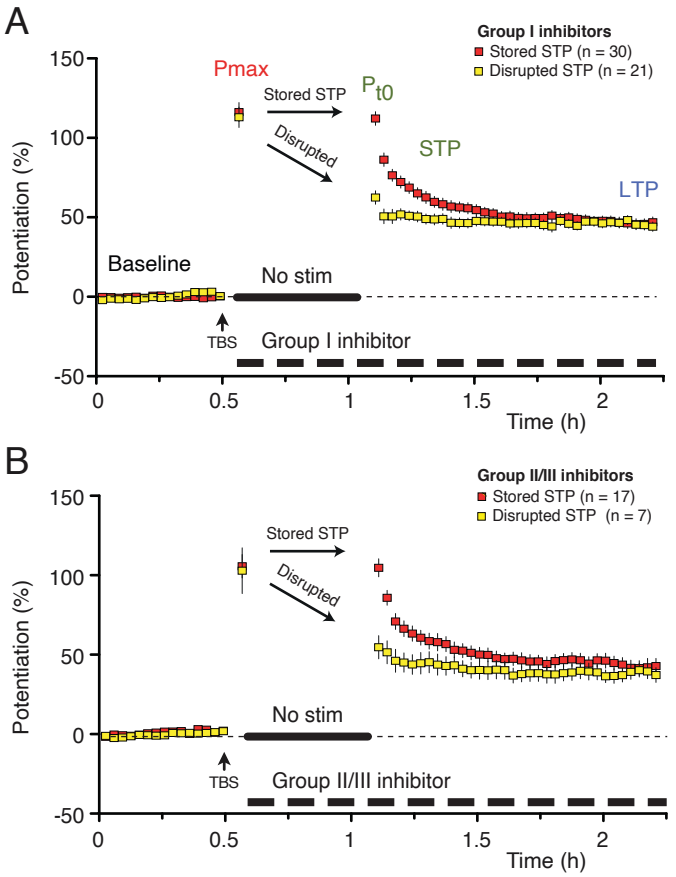

Fig.3 Promiscuous mGluRs; Ingram R & Volianskis A 2024

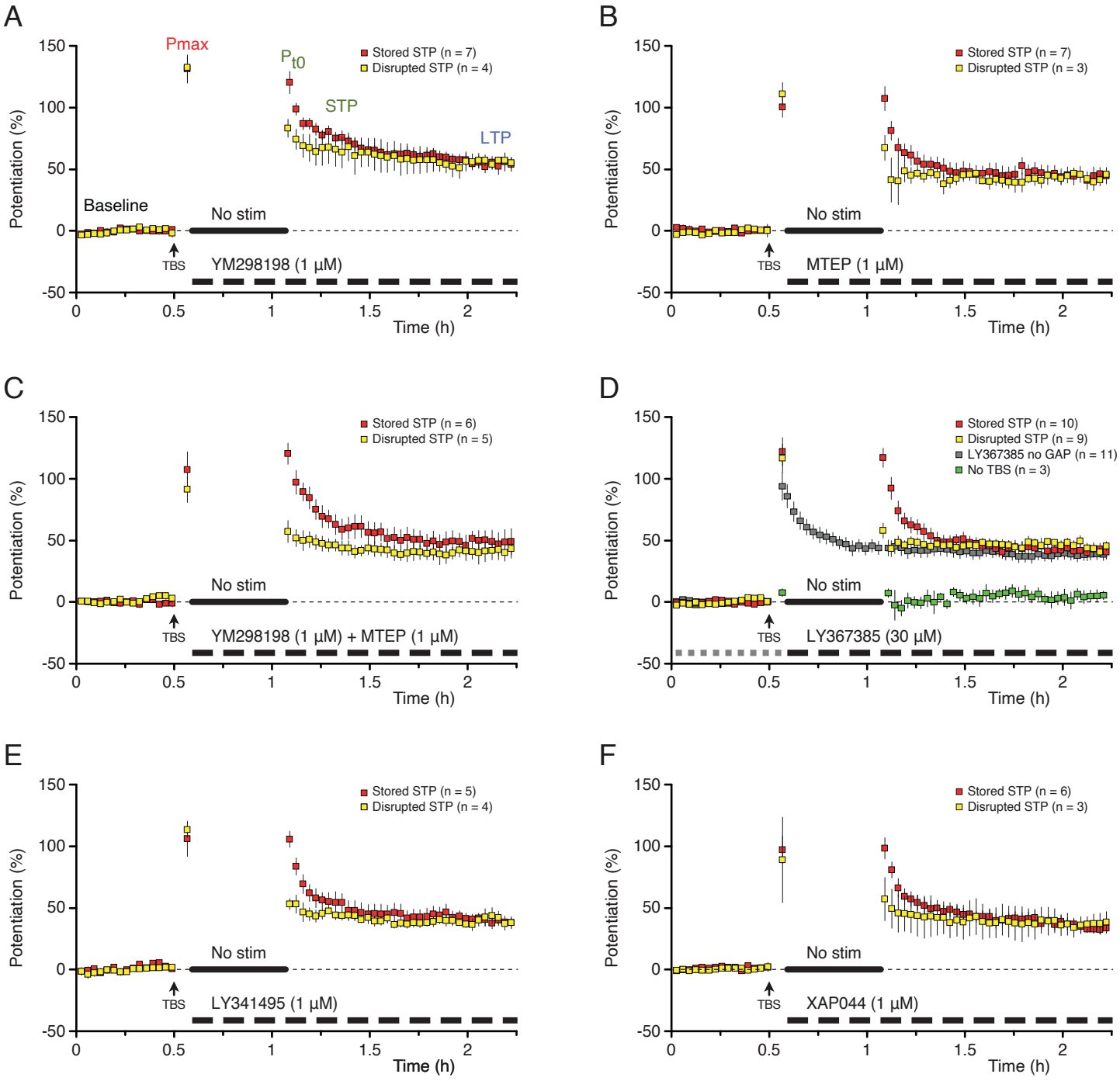

Table 1. Incidence of stored and disrupted NMDAR-STP across the animals used.

Number of animals	100 % stored STP	100 % disrupted STP	Stored & disrupted
1-slice exps (n = 37)	*n = 25 (67.6 %)	n = 12 (32.4 %)	---
2-slice exps (n = 19)	n = 10 (52.6 %)	n = 3 (15.8 %)	n = 6 (31.6 %)
3-slice exps (n = 5)	n = 3 (60.0 %)	n/o (0 %)	**n = 2 (40.0 %)
*Includes 6 controls without application of antagonists, **2 slices stored and 1 disrupted.			

Caption: 61 rats were used in the experiments (exps, n refers to animal numbers) with the 30-min pause in stimulation and multiple-slice recordings were performed in slices from 24 animals. In terms of the storage and disruption of NMDAR-STP a variety of outcomes were observed, showing no association with the animal identity (n/o means not observed). Thus, in some of the animals either only stored or only disrupted NMDAR-STPs were observed (100 % stored STP; 100 % disrupted STP) whilst in some other animals both stored and disrupted STPs were prevalent. The experiments were performed over a 2-year period and the observations were randomly spread-out over the time.