sessions, participants completed a range of tasks, including high- and low-g spatial (Fig. 1A); high- and low-g verbal (Fig. 1B); and two standard measures of g, Cattell’s Culture Fair, Scale 2 Form B (including high- and low-spatial 24/34 items correct, range 12 to 31). For the Scale 2 Form A, with one section omitted because of high-response rate (range 4 to 18); low-response rate (range 1 to 17); high-verbal, 7 (range 1 to 17); low-verbal, 41 (range 16 to 75).

PET was used to obtain regional cerebral bloodflow (rCBF) data in 13 right-handed participants, mean age 26 (range 21 to 34). PET imaging was approved by the Ethics Committee of Heinrich-Heine-Universität Düsseldorf (Application 868). Measurements were made with an eight-ring PET camera (PC4096 plus, GE/Scanditronix, Upplåsa, Sweden). The session included six scans (one for each of the tasks described in this report, plus one more of no relevance here), with the order of tasks approximately counterbalanced across participants. To allow for decay of radiation (19), successive scans were separated by 15 min, toward the end of which the 0.5-min practice period (see legend to Fig. 1) of the next task was given. For each scan, 1480 MBq of [15O]butanol was injected into the right brachial vein, flushed with 10 ml of saline. PET scanning and the 2-min task period (see legend to Fig. 1) began at the moment of the injection, so that the participant was fully engaged in the task when radioactivity reached the brain about 11 s later. As previously described (20), rCBF was calculated from the first 40 s of dynamically recorded head uptake data.

PET image slices were reconstructed with a Hanning filter to an effective image resolution (full width at half maximum, FWHM) of 9 mm with a slice thickness of 6.4 mm. Further analysis was undertaken with SPM99 (http://www.fil.ion.ucl.ac.uk/spm). Scans for each subject were realigned, spatially normalized onto the PET template, and smoothed with an isotropic Gaussian kernel with FWHM set at 16 mm. The SPM99 gray matter threshold was set to its default value. Task comparisons, an ANCOVA (analysis of covariance) model was fitted to the data for each voxel. To remove confounds due to head movement across scans, high-pass filters (21) were applied to 4D time course images to determine whether scan order or any of the six head movement parameters were significantly associated with rCBF values (22). Those parameters with significant associations (scan order, translation in x, rotation in x) were set as covariates of no interest, along with global blood flow. In this report we describe only those parameters with significant associations (high-g < low-g) in regions of motor and medial premotor cortex, reflecting the higher response rate in the low-g task, and bilaterally at the temporoparietal junction.


12. Mean values of estimated global blood flow (ml dl−1 min−1) for each task were as follows: high-g spatial, 49.4; low-g spatial, 49.9; high-g verbal, 49.8; low-g verbal, 49.7.

13. The circle tasks was included in the first behavioral study (9) and the PET session (10). In the behavioral study, mean score (number correct 4 min) was 87 (range 53 to 112). In PET, the mean estimated global blood flow (ml dl−1 min−1) was 49.4.


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The Role of GTP-Binding Protein Activity in Fast Central Synaptic Transmission

Tomoyuki Takahashi,* Tetsuya Hori, Yoshinao Kaijika, Tetsuhiro Tsujimoto

Guanosine 5′-triphosphate (GTP)–binding proteins (G proteins) are involved in exocytosis, endocytosis, and recycling of vesicles in yeast and mammalian secretory cells. However, little is known about their contribution to fast synaptic transmission. We loaded guanine nucleotide analogs directly into a giant nerve terminal in rat brainstem slices. Inhibition of G-protein activity had no effect on basal synaptic transmission, but augmented synaptic depression and significantly slowed recovery from depression. A nonhydrolyzable GTP analog blocked recovery of transmission from activity-dependent depression. Neither effect was accompanied by a change in presynaptic calcium currents. Thus, G proteins contribute to fast synaptic transmission by refilling synaptic vesicles depleted after massive exocytosis.
much slower (time constant, 15.7 s) than under control conditions (8.44 ± 1.2 s; n = 9, significantly different from both, P < 0.02) (Fig. 2B). To examine whether this effect is mediated by GTP sites or adenosine triphosphate (ATP) sites through cross talk, we loaded calyces with ATP (10 mM) together with GDPβS (3 mM). The recovery time constant was 13.5 ± 3.4 s (n = 3), which was not significantly different from that with GDPβS alone (P = 0.57). Thus, G proteins in the nerve terminals play a role in accelerating recovery from synaptic depression.

Both monomeric and heterotrimeric G proteins are potentially involved in accelerating recovery from depression. Mechanisms downstream of heterotrimeric G proteins such as cyclic nucleotide cascades may also be involved. When the intracellular cyclic nucleotide concentration was raised by bath application of forskolin (20 μM) in combination with isobutylmethylxanthine (200 μM), the amplitude of EPSCs increased (by 28 ± 3.6%, n = 7). After a 10-Hz train (0.3 s), EPSCs recovered with a mean time constant of 3.54 ± 0.30 s, similar to the control value (3.95 ± 0.30 s, P = 0.42 [compare with (15)]. Thus, cyclic nucleotides do not seem to be involved in recovery after depression.

Next we examined the effect of the nonhydrolyzable GTP analog guanosine 5′-O-(2-thiodiphosphate) (GTPγS). When GTPγS (0.2 mM) was infused into the calyx (Fig. 3A), EPSCs gradually diminished concomitantly with I_{Ca} and reached a low, steady level (35 ± 5%, n = 10) within 20 min (Fig. 3A) through activation of heterotrimeric G proteins (9, 18). A 10-Hz train administered in the presence of GTPγS (0.2 mM) depressed EPSCs (Fig. 3B) to 46 ± 5%, n = 9, a value between those obtained for the control (with GTP) and GDPβS (Fig. 1B). Although I_{Ca} was unchanged throughout, the recovery of EPSCs from depression was incompletely restored (13), indicating that the synaptic depression was caused by a mechanism downstream of Ca^{2+} influx (Fig. 1B). Because postsynaptic AMPA receptors recover from desensitization within 100 ms (14), the depression must be presynaptically mediated, most likely by the depletion of synaptic vesicles in RRP (15, 16), although reduction of release probability (17) may be an additional factor. When GTP (0.5 mM) was replaced by GDPβS (3 mM) in the presynaptic pipette, EPSCs were depressed to a greater extent (29 ± 7%; n = 6, P < 0.01) during the 10-Hz train (Fig. 1, B and C). At lower frequencies (0.2 to 1.0 Hz, 30 stimuli), the effect of GDPβS was not significant (Fig. 1C).

The recovery time from synaptic depression was measured from EPSC amplitude at various time intervals after the 10-Hz train (Fig. 2A). With GTP (0.5 mM) in the presynaptic pipette, the time constant of recovery was 3.32 s; whereas with GDPβS, recovery was much slower (time constant, 15.7 ± 2.6 s; n = 8, P < 0.001) (Fig. 2B). To exclude the possible side effect of GDPβS, we omitted GTP from presynaptic pipettes and washed out endogenous GTP for 20 min after rupture. The recovery time constant then fell to a value between those obtained for GTP and GDPβS (0.91 s; 2.6 s; n = 6).

![Fig. 1. Effects of GDPβS on basal synaptic transmission and depression. (A) EPSCs were evoked by presynaptic action potentials elicited by 1-ms depolarizing current pulse at 0.1 Hz. GDPβS (6 mM) was infused into the calyx (arrow). EPSCs and presynaptic action potentials before (1) and after (2) GDPβS application are shown above the panel. Bath application of baclofen (10 μM, 40 s) (filled bars) was done 10 min before and 10 s after GDPβS application. Sample records were averaged from six consecutive records in this and in subsequent figures. (B) EPSCs (lower panel) evoked by I_{Ca} (upper panel) underwent synaptic depression during a train of repetitive stimulation (10 Hz, 3s) and recovered after returning to 0.1 Hz. Synapses with their calyces were loaded either with GDPβS (3 mM) (●) or GTP (0.5 mM) (○). Amplitudes of I_{Ca} and EPSCs are normalized to their mean amplitude at 0.1 Hz before the 10-Hz train. I_{Ca} and EPSCs before (1), during (2), and after (3) the 10-Hz train are superimposed in sample records in the upper panel. (C) The frequency dependence of the depression ratio at calyces loaded with GTP (●) or GDPβS (○). Data points and error bars represent the mean ± SEM from five to eight synapses in this and in subsequent figures. The depression ratio was estimated from the mean amplitude of the last five EPSCs during the repetitive stimulation divided by the mean amplitude of five EPSCs in the control at 0.1 Hz. At six synapses, the difference between GTP and GDPβS is significant at 10, 5, and 2 Hz (P < 0.02), but insignificant at 1.05, and 0.2 Hz (P > 0.1). The mean amplitude of EPSCs (2.43 ± 0.54 nA, n = 6) in calyces loaded with GTP is comparable to that with GDPβS (2.04 ± 0.30 nA, n = 6).

![Fig. 2. Effects of guanine nucleotides on the time course of recovery from synaptic depression. (A) Experimental protocol and sample recordings of EPSCs during a 10-Hz train (left, superimposed) and of EPSCs evoked by test pulses at different time intervals (Δt, right, superimposed) after the conditioning train. (B) The time course of recovery with GTP (n = 4 to 6) (●), with GDPβS (n = 5 to 8) (○), or without guanine nucleotides (n = 8 to 9, 20 to 40 min after whole-cell recording) (Δ). Single exponential curves were fit by the least-squares method. The negative values for the first two points (0 and 0.5 s) with GDPβS may be due to the recovery from combined synaptic facilitation masked by depression during high-frequency stimulation. (C) Time course of recovery from depression in calyces loaded with GTPγS (0.2 mM, n = 6 to 8). The recovery time courses in calyces loaded with GTP or GDPβS are shown by dashed lines.](image-url)
complete in GTPγS (Fig. 3B) (88 ± 3%, n = 6) (19), indicating that the recovery of EPSCs from depression was blocked at the step downstream of Ca^{2+} influx. Despite the incomplete recovery of EPSCs from depression, the rate of recovery was similar to that for the control (GTP, P > 0.6), with a mean time constant of 3.82 ± 0.93 s (n = 7) (Fig. 2C). Thus, GTPγS can replace GTP with respect to the rate but not with respect to the magnitude of recovery.

Tetanic stimulation (100 Hz for 10 s) causes a marked depression of EPSCs accompanied by inactivation of $I_{Ca}$, which is followed by a gradual recovery owing largely to the recovery of $I_{Ca}$ from inactivation (20). In GTPγS, EPSCs no longer recovered from posttetanic depression, whereas $I_{Ca}$ recovered from inactivation with a normal time course (n = 6) (Fig. 4A). EPSCs recovered normally in GDPβS (3 mM) or GTP (0.5 mM) (Fig. 4B). This block of recovery by GTPγS was not associated with a change in quantal size, because the mean amplitude of miniature EPSCs (mEPSCs) was the same after tetanic stimulation (107 ± 8.2%, n = 6) (Fig. 4C). Thus, GTP hydrolysis is essential for EPSCs to recover from synaptic depression (21).

Our results indicate that G-protein activity is essential for recovery from synaptic depression. The rate of recovery from depression mainly reflects the rate of RRP replenishment (15, 16), which is accelerated by intracellular Ca^{2+} (22). In many cell systems, intracellular Ca^{2+} concentration is affected by inositol phosphates and their receptors, both of which can be up-regulated by heterotrimeric G proteins (23). However, at the calyx of Held, loading of inositol 1,4,5-trisphosphate (30 μM) into the nerve terminal had no effect on the EPSC amplitude or the frequency of mEPSCs (24).

The Release of Neural Transmitter Substances

References and Notes

11. Transverse slices (150 μm thick) of superior olivary complex were prepared from 14- to 17-day-old Wistar rats killed by decapitation under halothane anesthesia (9, 10). Using a 60× water immersion objective in an upright microscope, we viewed the calyx of Held and its target, the MNTB principal neuron, with a charge-coupled device camera. The standard artificial cerebrospinal fluid (aCSF) contained [in mM] 120 NaCl, 2.5 KCl, 1.25 NaHCO_3, 1.25 NaH_2PO_4, 2 CaCl_2, 1 MgCl_2, 10 glucose, 3 myo-inositol, 2 sodium pyruvate, 0.5 ascorbic acid, 4 lactic acid (pH 7.3, gassed with 5% CO_2 and 95% O_2). Bicuculline (10 μM) and strychnine (0.5 μM) were routinely included in the aCSF. The postsynaptic patch pipette contained [in mM] 110 CsCl, 30 CreaCl, 10 Hepes, 5
Abolition and Reversal of Strain Differences in Behavioral Responses to Drugs of Abuse After a Brief Experience

Simona Cabib,1 Cristina Orsini,1 Michel Le Moal,2 Pier Vincenzo Piazza*2

Inbred strains of mice are largely used to identify the genetic basis of normal and pathological behaviors. This report demonstrates that a moderate period of food shortage, an ecologically common experience, can reverse or abolish strain differences in behavioral responses to the abused psychostimulant amphetamine. The period of food shortage occurred when the animals were mature and was terminated before the administration of amphetamine. Strain differences in behavior appear highly dependent on environmental experiences. Consequently, to identify biological determinants of behavior, an integrated approach considering the interaction between environmental and genetic factors needs to be used.

Inbred strains of mice are increasingly utilized to identify biological determinants of normal and pathological behaviors. This report demonstrates that a moderate period of food shortage, an ecologically common experience, can reverse or abolish strain differences in behavioral responses to the abused psychostimulant amphetamine. The period of food shortage occurred when the animals were mature and was terminated before the administration of amphetamine. Strain differences in behavior appear highly dependent on environmental experiences. Consequently, to identify biological determinants of behavior, an integrated approach considering the interaction between environmental and genetic factors needs to be used.