action $a(j)$ and reward $r(j)$ ($j = 1, \ldots, i - 1$) (3, 22). The estimated action values successfully predicted the probability of subsequent action choices, $a(i)$. (Fig. 4A and fig. S2). Figure 4, B and C, shows a $Q_i$-type neuron whose discharge rate during the delay period followed the time course of $Q_i$ but not of $Q_{i-1}$ (double asterisk regression slope for $Q_i = -9.7, P < 0.001$, slope for $Q_{i-1} = 2.3, P = 0.29$) (Fig. 4, B and C). These results suggest that a large subset of striatal neurons encode the action values that are updated by the history of actions and rewards and determine the probability of selecting a particular action.

Action-value coding in the striatum may be a core feature of information processing in the basal ganglia. The striatum is the primary target of dopaminergic signals, which regulate the plasticity of cortico-striatal synaptic transmission (10, 11), conveying signals of actions and cognition. Thus, the striatum may be the locus where dopaminergic signals, which regulate the plasticity of cortico-striatal synaptic transmission, convey signals of actions and cognition.

References and Notes


Behavioral sensitization of motor activity induced by repeated noncontingent or self-administration of several drugs of abuse such as amphetamine, cocaine, heroin, and nicotine is an animal model of enduring drug-induced neuroplasticity (1–4). Behavioral sensitization involves neural adaptations in mesocorticlimbic regions, including the NAc, that receive dopaminergic (DA) projections from the ventral tegmental area (VTA) and excitatory glutamatergic inputs from the prefrontal cortex (PFC) (5, 6). Initial work on behavioral sensitization focused on pre- and postsynaptic changes in DA systems, but recent evidence implicates synaptic plasticity in glutamatergic transmission in both the VTA and NAc (6–9). Whereas experience-dependent alterations in synaptic strength in the VTA are linked to the induction of behavioral sensitization, synaptic plasticity in the NAc appears to mediate its long-term maintenance and expression (7, 10, 11). Recent experiments indicate a role for LTD, a proposed cellular substrate for learning and memory, in behavioral sensitization. Sensitized mice show an enhanced depression

Nucleus Accumbens Long-Term Depression and the Expression of Behavioral Sensitization

Karen Brebner, Tak Pan Wong, Lidong Liu, Yitao Liu, Paul Campsall, Sarah Gray, Lindsay Phelps, Anthony G. Phillips, Yu Tian Wang.*

Drug-dependent neural plasticity related to drug addiction and schizophrenia can be modeled in animals as behavioral sensitization, which is induced by repeated noncontingent or self-administration of many drugs of abuse. Molecular mechanisms that are critical for behavioral sensitization have yet to be specified. Long-term depression (LTD) of $\alpha$-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid receptor (AMPAR)–mediated synaptic transmission in the brain has been proposed as a cellular substrate for learning and memory. The expression of LTD in the nucleus accumbens (NAc) required clathrin-dependent endocytosis of postsynaptic AMPARs. NAc LTD was blocked by a dynamin-derived peptide that inhibited clathrin-mediated endocytosis or by a GluR2-derived peptide that blocked regulated AMPAR endocytosis. Systemic or intra-NAc infusion of the membrane-permeable GluR2 peptide prevented the expression of amphetamine-induced behavioral sensitization in the rat.

References


Behavioral sensitization of motor activity induced by repeated noncontingent or self-administration of several drugs of abuse such as amphetamine, cocaine, heroin, and nicotine is an animal model of enduring drug-induced neuroplasticity (1–4). Behavioral sensitization involves neural adaptations in mesocorticlimbic regions, including the NAc, that receive dopaminergic (DA) projections from the ventral tegmental area (VTA) and excitatory glutamatergic inputs from the prefrontal cortex (PFC) (5, 6). Initial work on behavioral sensitization focused on pre- and postsynaptic changes in DA systems, but recent evidence implicates synaptic plasticity in glutamatergic transmission in both the VTA and NAc (6–9). Whereas experience-dependent alterations in synaptic strength in the VTA are linked to the induction of behavioral sensitization, synaptic plasticity in the NAc appears to mediate its long-term maintenance and expression (7, 10, 11). Recent experiments indicate a role for LTD, a proposed cellular substrate for learning and memory, in behavioral sensitization. Sensitized mice show an enhanced depression
of excitatory glutamatergic transmission in the NAc after repeated cocaine applications (12), providing the first correlation between acquired LTD and experience-dependent neural plasticity mediated by addictive drugs. The molecular mechanisms underlying NAc LTD remain to be specified, and no specific inhibitor for LTD is available to test its causative role in the expression of behavioral sensitization.

Experiments with hippocampal brain slices have linked LTD to a specific reduction in synaptic transmission mediated by facilitated endocytosis of postsynaptic α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid receptors (AMPARs) (13–15). To determine whether a similar mechanism underlies NAc LTD, we performed whole-cell recordings of excitatory postsynaptic currents (EPSCs) in medium spiny neurons in the shell region of the NAc that were evoked by electrical stimulation of glutamatergic cortical inputs. LTD was induced by paired presynaptic stimulation (1 Hz, 480 pulses) with postsynaptic depolarization by holding membrane potential at −50 mV. (A) LTD was induced by pairing presynaptic stimulation (1 Hz, 480 pulses) with postsynaptic depolarization by holding membrane potential at −50 mV. (B and C) NAc LTD required facilitated clathrin-dependent endocytosis in the postsynaptic neuron. Inclusion in the patch pipette of a dynamin peptide (Dyn, 100 μg/ml, n = 8) prevented LTD (B), whereas the inclusion of a scrambled form of the dynamin peptide (scrambled Dyn, 100 μg/ml, n = 7) had little effect on LTD (C). (D and E) GluR2-dependent endocytosis of postsynaptic AMPARs is required for expression of NAc LTD. The application of GluR23Y (GluR23Y, 100 μg/ml, n = 14) (D), but not the control GluR23A (GluR23A, 100 μg/ml, n = 12) (E) peptide via recording pipettes abolished LTD expression. (F) Bar graphs summarize data shown in (A) to (E). The double asterisks indicate P < 0.01 versus control (Tukey-Kramer test). Error bars indicate SEM.
facilitate the intracellular delivery of the peptide, both the wild-type GluR23Y peptide and inactive control GluR23A peptide were fused to the cell membrane transduction domain of the HIV-1 Tat protein [YGRKKRRQRRR (24)] to generate Tat-GluR23Y and Tat-GluR23A using previously published methods (25). Tat-GluR23Y was transduced into neurons and blocked N-methyl-D-aspartate (NMDA)-induced AMPAR endocytosis in a neuronal cultured model of LTD (fig. S2) (26).

Fig. 2. Intravenous administration of the interference Tat-GluR23Y peptide blocks d-amphetamine-induced behavioral sensitization of stereotypy. (A and B) Transduction of Tat-GluR23Y peptide in rat brain. After iv injection of dansyl-Tat-GluR23Y peptide (1.5 nmol/g), animals were perfused with saline at indicated time points to remove residual peptide in the blood stream. The concentrations of peptide at different time points (A) were then determined with reference to a standard curve constructed with different known amounts of dansyl-Tat-GluR23Y peptide added into brain extract from control animals (B). (C and D) Systemic pretreatment of Tat-GluR23Y, but not Tat-GluR23A, prevented the sensitized behavioral response to a challenge dose of d-amphetamine in sensitized rats. Sensitized rats (n = 6 to 8 per group) were pretreated with Tat-GluR23Y or saline by iv injection (1.5 nmol/g) 90 min before a challenge dose of d-amphetamine (2 mg/kg, ip). In (C), stereotypy scores were assessed at 10-min intervals. Points represent mean stereotypy scores (mean ± SEM) for each group of rats tested over the 2-hour session. Chronic salinetreated rats served as controls. (D) shows a summary of changes in stereotypy scores across the 2-hour test session converted to the area under the curve for individual groups depicted in (C). (E) Neither Tat-GluR23A nor Tat-GluR23Y affected lever presses for food pellets on a fixed-ratio 2 schedule during 2-hour test sessions (n = 11). (F) Neither peptide had an effect on self-administration of d-amphetamine (0.2 mg/kg/infusion) delivered on a progressive ratio schedule. No significant differences in the break point measures for drug reward were observed after Tat-GluR23Y or Tat-GluR23A administered 90 min before the test session (n = 6). Error bars indicate SEM.

Fig. 3. Intracranial microinjection of Tat-GluR23Y into the NAc, but not the VTA, prevents the expression of behavioral sensitization. (A) The left panel histogram shows that bilateral intracranial microinjection of Tat-GluR23Y (15 pmol) into the NAc blocks sensitized responses to the challenge dose of d-amphetamine in sensitized rats. The image on the right shows reconstructions of histology sections illustrating injection sites in the NAc. (B) Example of the diffusion of the fluorescent dansyl-Tat-GluR23Y peptide within the NAc after intra-NAc injection. Schematic diagram (left) illustrates the peptide diffusion area shown in the fluorescent image (middle). The right panel shows peptide transduction in individual NAc neurons at high magnification. In this example, the peptide diffuses to both the shell and core regions. ac, anterior commissure. (C) Bilateral intracranial microinjection of the Tat-GluR23Y peptide into the VTA fails to affect d-amphetamine–induced behavioral sensitization. The asterisks indicate P < 0.05 versus acute amphetamine group. n = 5 to 8 in each group. Error bars in (A) and (C) indicate SEM.
We next examined the potential role of NAc LTD in mediating the expression of behavioral sensitization to psychostimulant drugs after intravenous (iv) injection of Tat-GluR2\textsubscript{3Y} or Tat-GluR2\textsubscript{3A} peptides. Using fluorescently tagged peptides, we detected the presence of the peptides in the brain after a single iv injection of 1.5 nmol of peptide per g of body weight (nmol/g). Brain concentrations of the peptide increased in a time-dependent manner, with a peak concentration reached 90 min after injection (Fig. 2, A and B). Behavioral sensitization was induced by 10 intraperitoneal (ip) injections of \(\alpha\)-amphetamine [2.0 mg drug per kg body weight (mg/kg)] on alternate days. The expression of behavioral sensitization was evoked by a challenge dose of \(\alpha\)-amphetamine (2.0 mg/kg, ip) 21 days later. Pretreatment of sensitized rats with iv Tat-GluR2\textsubscript{3Y} (1.5 nmol/g) 90 min before the challenge dose of \(\alpha\)-amphetamine prevented the expression of behavioral sensitization. Pretreatment with control Tat-GluR2\textsubscript{3A} or with saline had no effect (Fig. 2, C and D). Coadministration of Tat-GluR2\textsubscript{3Y} with an acute injection of \(\alpha\)-amphetamine did not significantly change (\(P > 0.05\)) stereotypy responses (Fig. 2, C and D) or locomotor scores (636.5 \pm 153.3, \(n = 6\)) relative to acute \(\alpha\)-amphetamine alone (563.8 \pm 94.7, \(n = 4\)). No noticeable behavioral side effects were associated with peptide pretreatment. The effects of Tat-GluR2\textsubscript{3Y} do not appear to represent a general disruption of learned motor behavior, because systemic treatment with Tat-GluR2\textsubscript{3Y} failed to disrupt operant responding for food pellets delivered on a fixed ratio–2 schedule of reinforcement (Fig. 2E). Similar treatment with Tat-GluR2\textsubscript{3Y} or Tat-GluR2\textsubscript{3A} did not affect motivation to respond for iv \(\alpha\)-amphetamine in a more demanding progressive ratio schedule of reinforcement (Fig. 2F), in which successive deliveries of drug reward require progressively more operant responses (27). To determine whether systemically applied Tat-GluR2\textsubscript{3Y} peptide disrupted the expression of behavioral sensitization by its action in the NAc but not the VTA, we performed intracranial microinfusion of Tat-GluR2\textsubscript{3Y} (15 pmol, 60 min before \(\alpha\)-amphetamine challenge) into the NAc or VTA, with Tat-GluR2\textsubscript{3A} or saline serving as controls. Consistent with our hypothesis, microinfusion of Tat-GluR2\textsubscript{3Y} directly into the NAc (Fig. 3, A and B), but not the VTA (Fig. 3C), prevented the expression of behavioral sensitization. Pretreatment with either Tat-GluR2\textsubscript{3A} or saline failed to influence \(\alpha\)-amphetamine-induced behavioral sensitization of stereotyped behavior. Synaptic plasticity at excitatory synapses has been proposed as the cellular substrate of information processing and memory formation in the brain under both physiological (28, 29) and pathological conditions, including addiction (7). Experiments characterizing the nature of synaptic plasticity in sensitized animals have observed both LTP (30) and LTD (16) in the NAc. Furthermore, increased excitatory drive in the NAc is observed during drug-seeking behavior (31). Within a given nucleus such as the NAc, it is suggested that the relative balance between LTP and LTD at different afferent inputs determines the expression of specific patterns of behavior (32). In sensitized animals, the abnormal induction of LTD at PFC inputs to the NAc may disrupt the influence of this pathway on neural activity involved in goal-directed behavior (32). However, as in any other area of the brain, exact roles of either NAc LTP or LTD in behavioral sensitization have not previously been defined because of the lack of specific inhibitors that block the expression of LTP or LTD without affecting any upstream signaling.

Here we identified clathrin-mediated and GluR2-dependent endocytosis of postsynaptic AMPARs as the final step in the expression of NAc LTD. We also demonstrated that the GluR2\textsubscript{3Y} peptide, which specifically blocks the endocytosis of postsynaptic AMPARs, prevented the expression of NAc LTD without affecting basal synaptic transmission and other upstream signaling processes involved in LTD. Using the membrane-permeant form of GluR2\textsubscript{3Y} peptide, we were able to probe the role of LTD in freely moving rats with great specificity and thereby provide evidence for the involvement of NAc LTD in the expression of behavioral sensitization. Because the GluR2\textsubscript{3Y} peptide used here has no effect on hippocampal CA1 LTP (23), a form of LTP involving regulated AMPAR insertion (15), the peptide appears not to affect the pool of AMPARs available for regulated insertion and hence does not affect the NAc LTP. Thus, determination of the exact role of NAc LTP in behavioral sensitization awaits the availability of an LTP-specific inhibitor.

Our work provides strong evidence for an essential role of NAc LTD in behavioral sensitization and thereby demonstrates the utility of peptides that disrupt the final step in the expression of synaptic plasticity as tools to examine the critical role of LTD and/or LTP in specific aspects of learning and memory in conscious animals. Given the links between behavioral sensitization and neuroplasticity induced by a potent psychostimulant drug of abuse, these data raise the possibility that treatment with new compounds, such as the GluR2\textsubscript{3Y} peptide used here, that disrupt psychostimulant-induced synaptic and behavioral plasticity may form the basis for a rational drug-development strategy for treating maladaptive neuroadaptations related to drug addiction.

References and Notes
17. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
23. Hippocampal CA1 LTP was induced in rat slice preparations by pairing Schaffer collateral stimulation (200 pulses stimulation at 2 Hz) with postsynaptic depolarization of the cell to −5 mV from a holding membrane potential of −60 mV. LTP was reliably induced in cells recorded with pipette solutions supplemented with or without GluR2\textsubscript{3Y} peptide [normalized EPSC amplitude: 164 ± 22% for GluR2\textsubscript{3Y} (100 \&micro;M, n = 7) versus 168 ± 18% for control (n = 7, P > 0.05)]. Thus, the GluR2\textsubscript{3Y} peptide appears to not affect LTP expression.
33. This work was supported by the Brain Repair Program of Neuroscience Canada (A.G.P. and Y.T.W.) and the Canadian Institutes of Health Research (CfHR). Y.T.W. is an international research scholar of the Howard Hughes Medical Institute and T.P.W. is a Research Fellow of CfHR. We gratefully acknowledge useful initial discussions with Y. Dong and advice from N. Gorelova for NAc slice preparation and from M. Thomas and A. Bonci on recording of NAc LTD in slices.