Severe Dopaminergic Neurotoxicity in Primates After a Common Recreational Dose Regimen of MDMA ("Ecstasy")

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The prevailing view is that the popular recreational drug (±)3,4-methylenedioxymethamphetamine (MDMA, or "ecstasy") is a selective serotonin neurotoxin in animals and possibly in humans. Nonhuman primates exposed to several sequential doses of MDMA, a regimen modeled after one used by humans, developed severe brain dopaminergic neurotoxicity, in addition to less pronounced serotonergic neurotoxicity. MDMA neurotoxicity was associated with increased vulnerability to motor dysfunction secondary to dopamine depletion. These results have implications for mechanisms of MDMA neurotoxicity and suggest that recreational MDMA users may unwittingly be putting themselves at risk, either as young adults or later in life, for developing neuropsychiatric disorders related to brain dopamine and/or serotonin deficiency.

MDMA ("ecstasy") has become a popular recreational drug internationally (*1*, *2*). In the 1980s, MDMA was generally used on college campuses, with most individuals taking no more than one or two 75- to 150-mg doses, about 1.6 to 2.4 mg per kilogram of body weight (mg/kg), twice monthly (*3*). More recently, MDMA is increasingly used in the context of large, all-night dance parties where partygoers regard the drug as safe and consume multiple doses during the night (*4*, *5*).

MDMA appears to carry risks beyond the sociobehavioral effects associated with drug abuse. Experimental animals treated with MDMA show evidence of brain serotonin neurotoxicity (*6*–*8*), and MDMA-induced serotonin neurotoxicity may also occur in humans (*9*, *10*). Virtually all animal species tested until now show long-term effects on brain serotonin neurons but no lasting effects on either brain dopamine or norepinephrine (NE) neurons (*6*–*8*). In the mouse, dopamine neurons are affected, but serotonin neurons are spared (*11*, *12*).

We used nonhuman primates to evaluate the neurotoxic potential of a dose regimen modeled closely after one often used by MDMA users at all-night dance parties. Squirrel monkeys (*Saimiri sciureus*) were given MDMA at a dosage of 2 mg/kg, three times, at 3-hour intervals, for a total dose of 6 mg/kg (*13*). Of five monkeys treated with MDMA, three tolerated drug treatment without any apparent difficulty. One monkey became less mobile and had an unstable, tentative gait after the second dose, and therefore it was not given the third planned dose. The fifth monkey developed malignant hyperthermia and died within hours of receiving the last dose of MDMA. Two weeks after MDMA treatment, the three monkeys that tolerated drug treatment were examined for chemical and anatomic markers of brain serotonin neurons (*13*), along with three saline-treated control animals. These studies revealed lasting reductions in regional brain serotonin, serotonin's major metabolite (5-hydroxyindoleacetic acid, or 5-HIAA), and the serotonin transporter (SERT). Anatomic studies (*13*) supported these observations, showing reductions in the density of serotonin- and SERT-immunoreactive (SERT-IR) axons in some cortical regions (Fig. 1). Six weeks after MDMA treatment, the monkey that received only two doses of MDMA was evaluated and found to also have long-lasting reductions in serotonin axonal markers; serotonin, 5-HIAA, and SERT in the caudate nucleus of this animal were reduced by 37, 48, and 40%, respectively.

These same monkeys had marked reductions in various markers of striatal dopami-

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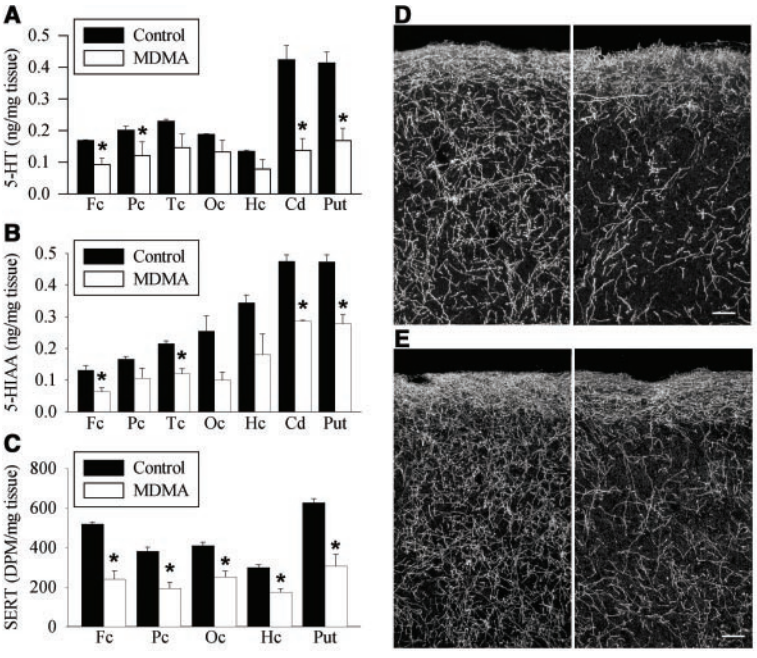

Fig. 1. Effect of MDMA on regional brain (**A**) serotonin (5-HT), (**B**) 5-HIAA, and (**C**) SERT in squirrel monkeys 2 weeks after drug treatment. Results shown represent the mean ± SEM (n = 3 animals per group). DPM, disintegrations per minute; Fc, frontal cortex; Pc, parietal cortex; Tc, temporal cortex; Oc, occipital cortex; Hc, hippocampus; Cd, caudate nucleus; Put, putamen. Asterisk designates $P < 0.05$, determined by individual comparison to control after one-way analysis of variance showed an F value with $P < 0.05$. (**D**) 5-HT– and (**E**) SERT-IR axons in the parietal cortex of a control monkey (left) and a monkey treated with MDMA 2 weeks previously (right). Dark-field photomicrographs of the coronal plane are shown; scale bar = 100 μm. (**F**) radioisotope [^{3}H]RTI-55–labeled SERT in coronal section of a control monkey (CON) and a monkey treated with MDMA 2 weeks previously. The scale on the right shows the density of binding sites designated by color expressed in nanocuries (nCi) per mg of tissue.

nergic axons (Fig. 2). The profound loss of striatal dopaminergic axonal markers was consistently observed in all monkeys examined, including the animal that received only two MDMA doses; dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), and the dopamine transporter (DAT) in the caudate nucleus of this animal were reduced by 65, 77, and 51%, respectively, 6 weeks after MDMA exposure. The loss of dopaminergic axonal markers was greater than the loss of serotonergic axonal markers. Morphologic studies revealed corresponding reductions in the density of striatal DAT- and tyrosine hydroxylase (TH)–IR axons throughout the striatal complex, with some sparing of the more caudal portion of the caudate nucleus (Fig. 2). Quantitative autoradiography studies (*13*) confirmed the severe reductions in striatal DAT density (Fig. 2).

To determine whether the severe long-lasting decrements in dopaminergic axonal markers in squirrel monkeys were unique to this primate species, we tested the effects of the same MDMA regimen in baboons (*Papio anubis*) (*13*). Again, one of five animals died, this time shortly after receiving only two doses of MDMA. Malignant hyperthermia (up to 41.6°C) was again an important factor. A second baboon appeared unstable after the second dose of MDMA and therefore received only two of the three planned doses. Two to 8 weeks after treatment, the four surviving MDMA-treated baboons, along with three saline-treated control animals, underwent chemical and anatomic studies of brain dopamine and serotonin neurons (*13*). Neurochemical and quantitative autoradiography studies again revealed a profound loss of striatal dopaminergic axonal markers (Fig. 3). Dopaminergic deficits in the striatum of the baboon that received only two MDMA doses were as severe as those in the baboons that received all three doses. Baboons also developed less severe, but significant, long-term reductions in regional brain serotonergic neuronal markers (Fig. 3).

To evaluate the selectivity of the observed effects, we assessed the status of noradrenergic neurons in both monkeys and baboons. MDMA produced no long-term effects on NE levels or the density of NE transporters in the brain of either primate species (figs. S1 and S2). Consistent with the lack of a long-term effect of MDMA on the concentrations of NE and its transporter, the density of TH-IR axons in the cerebral cortex of MDMA-treated monkeys was unaffected (fig. S1).

To determine that the lasting loss of chemical and anatomic markers of striatal dopaminergic and serotonergic axons and axon terminals was, in fact, due to a neurotoxic insult rather than to lingering acute pharmacological effects of MDMA, we used Fink and Heimer's method (*14*), which allows for selective silver impregnation of degenerating axons and axon terminals. A monkey treated with MDMA and evaluated 3½ days later (*13*) had dense argyrophilic debris characteristic of axon terminal degeneration in the striatum (Fig. 4). No such degenerative debris was evident in the striatum of the control animal. We also found a vigorous glial response (Fig. 4) in adjacent striatal tissue sections processed for glial fibrillary acidic protein (GFAP) immunocytochemistry (*13*).

We next explored the possibility that monkeys with MDMA-induced dopaminergic neurotoxicity (with no evidence of Parkinsonism) are at increased risk for the development of motor dysfunction secondary to dopamine depletion (*13*). Monkeys ($n = 3$) received a challenge dose regimen of alpha-methyl-para-tyrosine (AMPT) 1 week before and 1 week after MDMA treatment. Using a dosage regimen of AMPT that gradually reduces brain dopamine concentrations, we hoped to model the progressive decline in brain dopaminergic function that occurs with normal aging (*15*). Compared to their baseline, monkeys were more sensitive to AMPT-induced motor dysfunction 1 week after MDMA treatment (fig. S3).

We report severe, functionally significant dopaminergic neurotoxicity, along with more modest serotonergic neurotoxicity, in primates treated with doses of MDMA modeled after those commonly used by recreational MDMA users. Earlier studies in nonhuman primates have generally involved administration of higher MDMA doses (5 or 10 mg/kg) twice daily (morning and evening) for 4 consecutive days. These dosage regimens typically engendered more severe but highly selective toxicity toward brain serotonin neurons, with no long-term effects on brain dopamine neurons (*16–18*). Because the drug regimens used in previous studies did not model those used by most MDMA users, the possibility remained that occasional MDMA

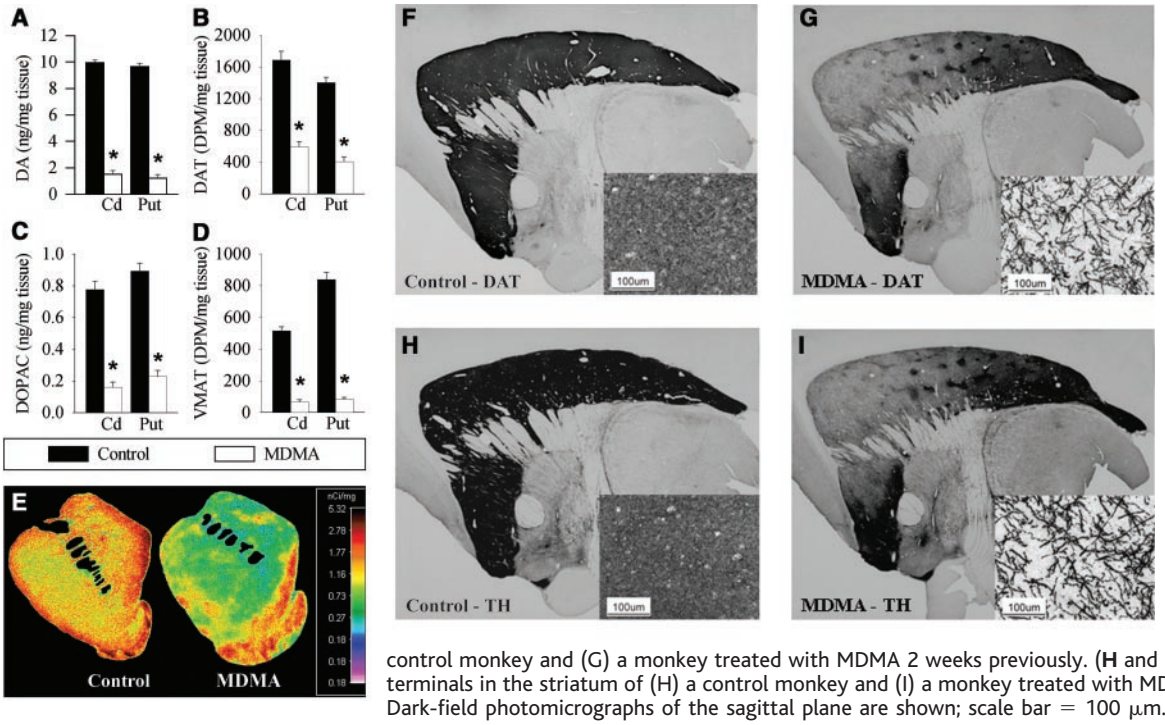

Fig. 2. Effect of MDMA treatment on striatal concentrations of (**A**) dopamine (DA), (**B**) [^{3}H]WIN35,428–labeled DAT, (**C**) DOPAC, and (**D**) radioisotope [^{3}H]MTBZ–labeled vesicular monoamine transporter-2 (VMAT) in squirrel monkeys examined 2 weeks after MDMA treatment. (**E**) [^{3}H]RTI-121–labeled DAT in coronal section of a control monkey and a monkey treated with MDMA 2 weeks previously. The scale on the right shows the density of binding sites designated by color expressed in nCi/mg of tissue. (**F** and **G**) DAT-IR axons and axon terminals in the striatum of (F) a control monkey and (G) a monkey treated with MDMA 2 weeks previously. (**H** and **I**) TH-IR axons and axon terminals in the striatum of (H) a control monkey and (I) a monkey treated with MDMA 2 weeks previously. Dark-field photomicrographs of the sagittal plane are shown; scale bar = 100 μm.

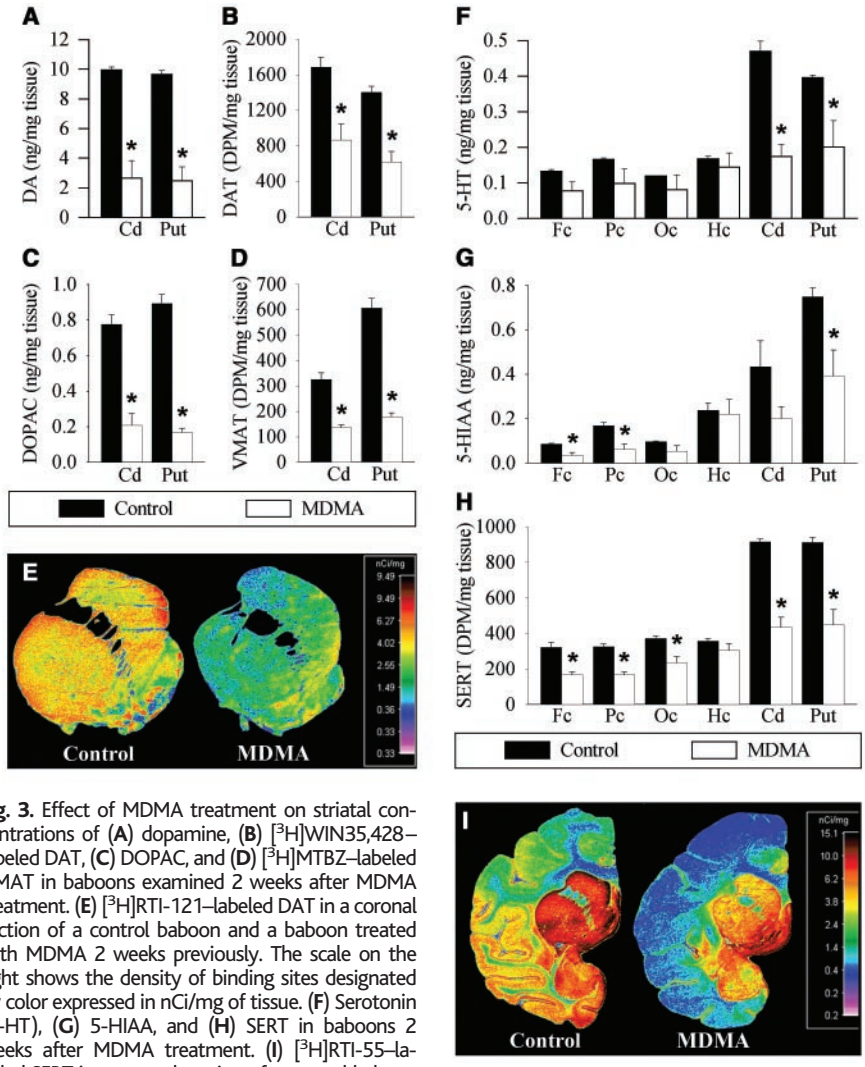

Fig. 3. Effect of MDMA treatment on striatal concentrations of (**A**) dopamine, (**B**) [^{3}H]WIN35,428–labeled DAT, (**C**) DOPAC, and (**D**) [^{3}H]MTBZ–labeled VMAT in baboons examined 2 weeks after MDMA treatment. (**E**) [^{3}H]RTI-121–labeled DAT in a coronal section of a control baboon and a baboon treated with MDMA 2 weeks previously. The scale on the right shows the density of binding sites designated by color expressed in nCi/mg of tissue. (**F**) Serotonin (5-HT), (**G**) 5-HIAA, and (**H**) SERT in baboons 2 weeks after MDMA treatment. (**I**) [^{3}H]RTI-55–labeled SERT in a coronal section of a control baboon and a baboon treated with MDMA 2 weeks previously. The scale on the right shows the density of binding sites designated by color expressed in nCi/mg of tissue.

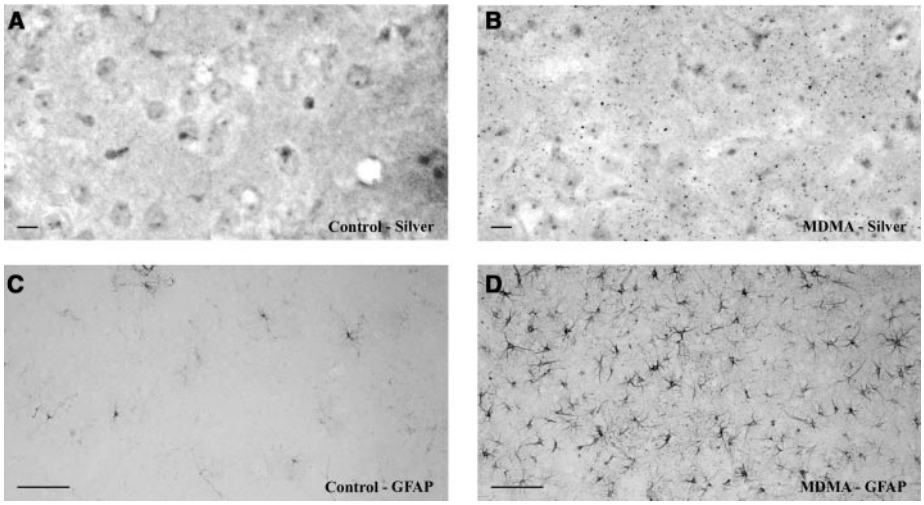

Fig. 4. Silver-stained coronal sections through the caudate nucleus of (**A**) a control monkey and (**B**) a monkey treated with MDMA (one dose of 2 mg/kg at 3-hour intervals, three times) 3½ days previously. Fine argyrophilic debris in the MDMA-treated monkey is characteristic of axon terminal degeneration, as demonstrated by the Fink-Heimer method (*14*). Scale bar = 10 μm. (**C**) Paucity of GFAP-IR cells in the caudate nucleus of a control monkey and (**D**) marked increase in the number of GFAP-IR cells in the striatum of a monkey treated with MDMA 3½ days previously. Scale bar = 10 μm.

users might not be at risk for neurotoxic injury. The present results, however, indicate that even individuals who use MDMA on one occasion may be at risk for substantial brain injury if they use two or three sequential doses, hours apart, as is often the case in recreational settings.

In the present studies, MDMA was given by a systemic route (subcutaneously in squirrel monkeys and intramuscularly in baboons), whereas humans generally take MDMA orally. It is possible that humans are at a decreased risk for neurotoxic injury because of differences in the route of administration. However, in the case of MDMA, oral administration offers little or no significant neuroprotection (*19–22*). Even if some degree of protection were afforded by oral administration, the profound loss of dopaminergic neuronal markers seen in both primate species suggests that significant neurotoxicity would still occur. Moreover, individual doses of MDMA used in this study are lower than those typically used by humans (1.6 to 2.4 mg/kg), once adjusted with interspecies dose scaling methods (*23*). Hence, any protection that might be associated with oral administration would likely be offset by increasing the dose of MDMA used in this study to the human equivalent. It is not uncommon for recreational MDMA users to use repeated doses of the drug on more than one occasion or more than two or three repeated doses per session.

The present findings challenge the commonly held notion that MDMA is a selective brain serotonin neurotoxin and carry important public health and scientific implications. Based on MDMA use pattern, there may be two separate MDMA cohorts: those with selective brain serotonergic neurotoxicity and those with combined serotonergic and more severe dopaminergic neural injury. It will be exceedingly important to consider this when attempting to identify and interpret functional consequences of MDMA use in humans. Cognitive abnormalities identified in MDMA users (*24–26*) may be related, at least in part, to dopaminergic rather than serotonergic neurotoxicity. The present findings also have implications for efforts aimed at identifying the mechanisms of MDMA neurotoxicity. Previous studies have identified a metabolite of MDMA that might be responsible for its neurotoxic effects, the 6-hydroxydopamine analog 2-(methylamino)-1-(2,4,5-trihydroxyphenyl) propane (*27–29*). Because this toxic metabolite induced both dopaminergic and serotonergic neurotoxicity, and because MDMA was believed to be a selective serotonin neurotoxin, it received little further attention. This 6-hydroxydopamine analog of MDMA obviously warrants closer scrutiny as a potential mediator of MDMA neurotoxicity.

The development of profound dopaminergic neurotoxicity after two or three sequential

MDMA doses of 2 mg/kg each leads one to question what distinguishes this particular drug regimen from the 4-day, twice daily, higher-dose regimen that engenders selective serotonergic neurotoxicity (*16*–*22*). One possibility is that the nonlinear pharmacokinetic profile of MDMA, such as that demonstrated in humans in the setting of closely spaced repeated dosing (*30*, *31*), leads to prolonged elevated brain levels of MDMA (or its metabolites) and that protracted exposure to MDMA renders dopamine neurons vulnerable to its toxic effects. An alternative (although not mutually exclusive) explanation is that repeated closely spaced doses of MDMA lead to higher elevations in body temperature, which is known to augment MDMA neurotoxicity (*32*). Additional studies are needed to evaluate these possibilities, in addition to alternative hypotheses.

In light of the present findings, and given the fact that MDMA use is widespread and increasing, one might ask why more cases of MDMA-induced Parkinsonism (*33*) have not been reported. There are multiple potential explanations, but only two will be mentioned. First, Parkinsonism does not generally become clinically apparent until more than 70 to 80% of brain dopamine has been depleted. Therefore, substantial MDMA-induced dopaminergic neurotoxicity could occur yet remain occult until unmasked by other processes (such as drug-induced interference with dopaminergic neurotransmission or decline in brain dopamine with advancing age). Second, until now, the potential for MDMA to damage brain dopamine neurons in primates has not been appreciated and, therefore, MDMA neurotoxicity has not been considered in the differential diagnosis of Parkinsonism in young adults. It is possible that some of the more recent cases of suspected young-onset Parkinson's disease might be related to MDMA exposure but that this link has not been recognized.

These findings suggest that humans who use repeated doses of MDMA over several hours are at high risk for incurring severe brain dopaminergic neural injury (along with significant serotonergic neurotoxicity). This injury, together with the decline in dopaminergic function known to occur with age (*15*), may put these individuals at increased risk for developing Parkinsonism and other neuropsychiatric diseases involving brain dopamine/serotonin deficiency, either as young adults or later in life.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/297/5590/2260/DC1
Materials and Methods
Figs. S1 to S3
References and Notes

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Conversion of Unc104/KIF1A Kinesin into a Processive Motor After Dimerization

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Unc104/KIF1A belongs to a class of monomeric kinesin motors that have been thought to possess an unusual motility mechanism. Unlike the unidirectional motion driven by the coordinated actions of the two heads in conventional kinesins, single-headed KIF1A was reported to undergo biased diffusional motion along microtubules. Here, we show that Unc104/KIF1A can dimerize and move unidirectionally and processively with rapid velocities characteristic of transport in living cells. These results suggest that Unc104/KIF1A operates in vivo by a mechanism similar to conventional kinesin and that regulation of motor dimerization may be used to control transport by this class of kinesins.

Caenorhabditis elegans Unc104 and the mouse ortholog KIF1A are kinesin motors that transport synaptic vesicle precursors along microtubules from the neuronal cell body to the nerve terminal (*1*–*3*). For such long-range transport to be efficient, organelles that encounter a microtubule must move processively. Conventional kinesin, which belongs to a different subfamily of vesicle-transporting kinesins, is dimeric and uses its two motor domains in a coordinated manner to take successive, unidirectional 8-nm steps along the microtubule without dissociating (*4*). However, KIF1A (*2*) and Unc104 (*5*) are monomeric in solution and are thought to operate using a different motility mechanism, because a single KIF1A motor domain has been shown to undergo biased diffusional movement along the microtubule (*6*). A novel processivity mechanism was proposed that involves an electro-

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