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Ventral Tegmental Area BDNF Induces an Opiate-Dependent–Like Reward State in Naïve Rats

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colloidal liquids (*19*). Bleaching experiments in early one-cell embryos revealed that components of these P granules (≤1 μm diameter) typically turned over in less than 30 s (fig. S7), suggesting that P granule droplets exist in dynamic equilibrium with soluble components, much as conventional liquids do.

Surface tension is another important parameter controlling the behavior of liquid drops. To quantify P granule surface tension, we measured the time scale of P granules undergoing both fusion and droplet breakup events (Fig. 3, A and B). This time scale increased approximately linearly with the droplet size, as expected for liquids (Fig. 3C). The slope of this line establishes the ratio of viscosity to surface tension, η/γ (*9*). Thus, together with our estimate of P granule viscosity ($\eta \approx 1$ Pa·s), we could make an order of magnitude estimate of the surface tension, γ, between P granules and the cytoplasm, $\gamma \sim 1$ μN/m, which is smaller than the air-water surface tension by a factor of $\sim 10^5$. Such small values are expected for macromolecular liquids (*9*); for example, colloidal liquids typically have $\gamma \sim 0.1$ μN/m and below (*19*, *20*). The surface energetic barrier to P granule formation thus should be small, which may explain their ability to rapidly form upon symmetry breaking (*9*).

The dissolution and condensation behavior we observed, together with the liquid-like nature of P granules, supports a simple physical picture for P granule localization based on the theory of demixing phase transitions in fluids (*21*). Before symmetry breaking, the concentration at which soluble P granule components are saturated, C_{sat}, is uniform and high; the concentration of soluble P granule components, C_{cyt}, is lower than this; P granules are thus undersaturated, causing dissolution throughout the embryo, similar to evaporation of water droplets at high temperature (Fig. 4, A and B). Symmetry breaking causes the posterior value of C_{sat} to decrease below C_{cyt}, and the posterior becomes supersaturated with P granule components; thus, P granule droplets begin condensing, much as air becomes supersaturated with water vapor as temperature is decreased, and water droplets condense. This posterior condensation occurs even while the anterior remains undersaturated, and anterior P granules continue dissolving. This gives rise to a diffusive flux of P granule components into the posterior, thereby clearing out anterior components. The gradient in the condensation point, C_{sat}, along the AP axis appears to be set by gradients in polarity proteins, including MEX-5 and PAR-1 (Fig. 4, C and D). Although protein degradation may also play a role, it appears here that polarity proteins function largely by spatially regulating the P granule "dew point."

We propose that P granule localization exemplifies a general mechanism for organizing the cytoplasm that arises from collections of weakly "sticky" molecules, including other ribonucleoprotein assemblies (e.g., P bodies, Cajal bodies, or stress granules) (*16*, *22*). Such phase structuring may represent a primordial mechanism for functional self-assembly of relatively unevolved molecular assemblies in the early stages of the evolution of life.

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Materials and Methods
SOM Text
Figs. S1 to S8
References
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Ventral Tegmental Area BDNF Induces an Opiate-Dependent–Like Reward State in Naïve Rats

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The neural mechanisms underlying the transition from a drug-nondependent to a drug-dependent state remain elusive. Chronic exposure to drugs has been shown to increase brain-derived neurotrophic factor (BDNF) levels in ventral tegmental area (VTA) neurons. BDNF infusions into the VTA potentiate several behavioral effects of drugs, including psychomotor sensitization and cue-induced drug seeking. We found that a single infusion of BDNF into the VTA promotes a shift from a dopamine-independent to a dopamine-dependent opiate reward system, identical to that seen when an opiate-naïve rat becomes dependent and withdrawn. This shift involves a switch in the γ-aminobutyric acid type A ($GABA_A$) receptors of VTA GABAergic neurons, from inhibitory to excitatory signaling.

The ventral tegmental area (VTA) serves as an anatomical locus controlling the switch from an opiate-nondependent to an opiate-dependent state (*1*, *2*). In nondependent rats, opiate reward is mediated by a dopamine-independent neural system, involving the brainstem tegmental pedunculopontine nucleus (TPP) (*3*). Once chronically exposed to opiates and in a state of withdrawal, opiate reward switches to a dopamine-dependent system (*3*). It has been observed that the switch between the two motivational systems is due to a switch in γ-aminobutyric acid type A ($GABA_A$) receptor functioning in VTA GABAergic neurons, from an inhibitory to an excitatory signaling state (fig. S1) (*2*).

Brain-derived neurotrophic factor (BDNF) is capable of producing this change in GABAergic response, from inhibitory to excitatory, as has been observed in the hippocampus during epileptic seizures (*4*) and in the spinal cord during neuropathic pain (*5*). BDNF is present in the VTA (*6*), and its TrkB receptors are present on both GABA (fig. S2) and dopamine VTA neurons (*7*, *8*). Chronic exposure to drugs of abuse increase BDNF levels in VTA neurons

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(*6*). Furthermore, BDNF infusions into the VTA dramatically enhance several behavioral effects of drugs, including psychomotor sensitization (*6*, *9*) and drug seeking (*6*, *10*). We hypothesized that, along with the changes in structural plasticity induced by BDNF in VTA dopaminergic neurons (*11*), increasing BDNF levels in the VTA would induce a switch to a drug-dependent motivational state in drug-nondependent rats due to the effects of BDNF on GABAergic neurons.

First, we examined whether BDNF protein and mRNA levels in the VTA were increased in opiate-dependent rats. Sixteen hours after withdrawal from repeated daily exposure to heroin (0.5 mg/kg, subcutaneously) for 8 days [see supporting online material (SOM)], BDNF protein ($F_{3,37} = 7.63$, $P < 0.05$) and BDNF mRNA ($F_{3,19} = 4.04$, $P < 0.05$) levels in the VTA increased by 150% ($P < 0.05$) and 193%, respectively, of the control drug-naïve rats ($P < 0.05$). However, there were no increases in BDNF either when rats received a single injection of heroin ($P > 0.05$) or 15 days after withdrawal from repeated heroin exposure ($P > 0.05$) (fig. S3).

To explore whether BDNF alone was sufficient to cause a change in the neurobiological substrates mediating opiate reward, we next performed place conditioning procedures on rats after single bilateral intra-VTA BDNF (0.25 μg each) infusions (*10*). Place conditioning procedures (*12*) (see SOM) were performed 3 days after BDNF infusions (*10*). During training, we administered four alternating injections of intraperitoneal morphine (10 mg/kg) and vehicle to the rats over 8 days. The dopamine receptor antagonist alpha-flupenthixol (0.8 mg/kg) (or its saline vehicle) was injected intraperitoneally 2.5 hours before each conditioning trial. Testing was performed drug-free between 48 and 72 hours after the final conditioning session. After VTA BDNF ($n = 10$ animals) [but not the saline vehicle phosphate-buffered saline (PBS) ($n = 20$)] infusion, antagonism of the dopaminergic system blocked the rewarding effects of acute systemic morphine in drug-nondependent rats ($F_{1,59} = 5.3$, $P < 0.05$, interaction of morphine and BDNF treatment). Infusion of BDNF outside of the VTA (due to cannulae misplaced rostral, ventral, or lateral to the VTA) (fig. S4) did not allow the dopamine antagonist to block the rewarding effects of morphine in nondependent rats ($t_{10} = 4.65$, $P < 0.05$, $n = 11$) (Fig. 1A). It is possible that intra-VTA BDNF modifies the ability of opiates to produce conditioned place preferences, making them easier to block. However, neither BDNF ($n = 7$) nor PBS ($n = 7$) alone had any effect on the sizes of the conditioned

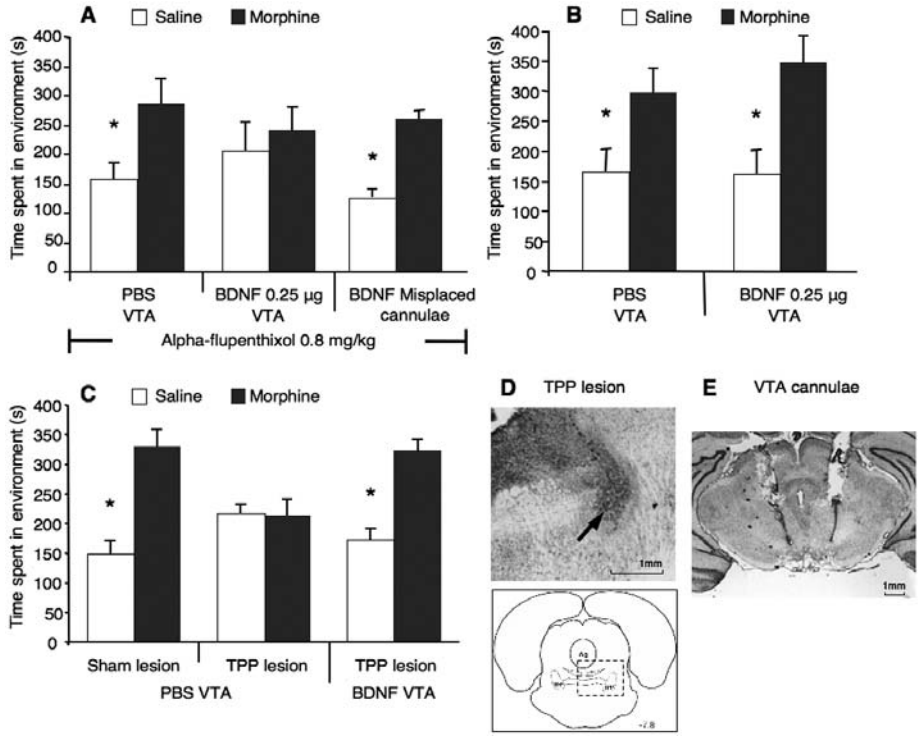

Fig. 1. Motivational effects of a single intra-VTA BDNF infusion in drug-nondependent rats. (**A**) Blockade of the dopaminergic system with neuroleptics (alpha-flupenthixol, 0.8 mg/kg) blocked the rewarding effects of morphine (10 mg/kg) in nondependent rats after a single intra-VTA BDNF (0.25 μg) infusion, but not after intra-VTA PBS infusion (*$P < 0.05$). The same treatment failed to block the rewarding effects of morphine in nondependent rats when BDNF was infused rostral, ventral, or lateral to the VTA because of missed cannulae placements (*$P < 0.05$). Error bars indicate SE of the mean. (**B**) Intra-VTA BDNF alone did not affect the sizes of the conditioned place preferences produced by acute morphine administration in drug-nondependent rats (*$P < 0.05$). (**C**) BDNF restored the rewarding properties of acute morphine administration in drug-nondependent TPP-lesioned rats (*$P < 0.05$). (**D**) Cresyl violet–stained coronal section showing one side of a bilateral TPP lesion and (below) a schematic of the anatomical region from which the section displayed was taken. The arrow indicates the TPP lesion area. (**E**) Cresyl violet–stained coronal section of a typical bilateral intra-VTA cannula placement.

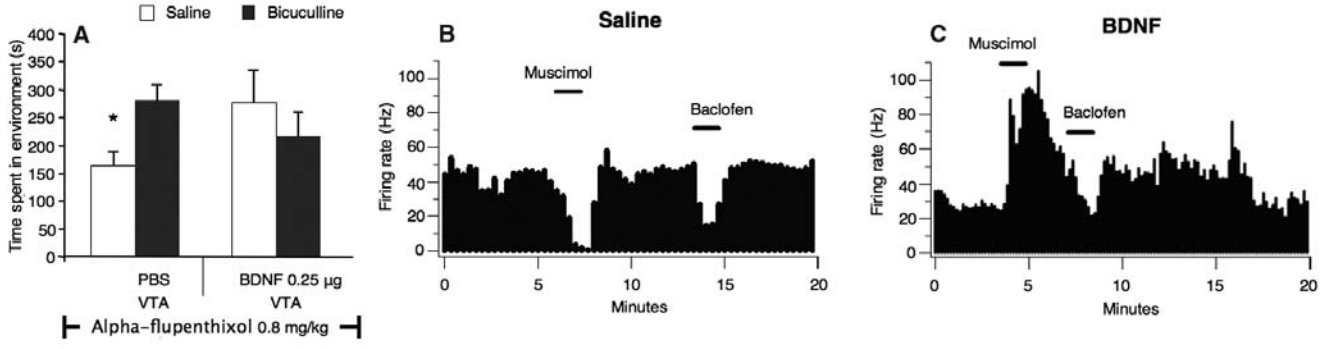

Fig. 2. Motivational and electrophysiological effects of a single intra-VTA BDNF infusion in drug-nondependent rats. (**A**) Blockade of the dopaminergic system with alpha-flupenthixol blocked the rewarding effects of an intra-VTA–administered $GABA_A$ receptor antagonist bicuculline (50 ng) in nondependent rats after a single intra-VTA BDNF infusion, but not after intra-VTA PBS infusion (*$P < 0.05$). (**B** and **C**) Single-unit extracellular recordings of VTA GABAergic neurons showing the effects of two current applications of muscimol (+50 nA) and baclofen (+50 nA) on the firing rate of representative VTA GABAergic neurons. In saline-treated rats, muscimol always decreased the firing rate of the VTA GABAergic neurons, whereas in BDNF-treated rats, muscimol enhanced the firing rate. Conversely, in both saline-treated and BDNF-treated rats, baclofen moderately decreased the firing rate of these VTA GABA neurons, as in this example.

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place preferences produced by morphine [$F_{1,27}$ = 12.84, $P < 0.05$, main effect of the morphine treatment only in both BDNF ($P < 0.05$) and PBS ($P < 0.05$) groups] (Fig. 1B).

To investigate whether BDNF in the VTA changes opiate reward from a dopamine-independent (TPP-dependent) to a dopamine-dependent reward system, we examined the effects of intra-VTA BDNF in TPP-lesioned rats (*13*, *14*). In drug-nondependent rats, bilateral TPP (n = 12) [but not sham (n = 14)] lesions blocked the rewarding properties of morphine ($F_{1,51}$ = 14.29, $P < 0.05$, interaction of lesion and morphine treatment). However, after BDNF infusion (n = 12), TPP lesions did not block morphine reward ($P < 0.05$) in drug-nondependent rats, similar to sham-lesioned rats, where morphine also caused reward ($P < 0.05$) ($F_{1,51}$ = 47.87, $P < 0.05$, main effect of morphine only), but opposite to TPP-lesioned rats infused with PBS, where morphine reward was blocked ($P < 0.5$) ($F_{1,47}$ = 17.14, $P < 0.05$, interaction of BDNF and morphine treatment) (Fig. 1C).

To examine whether the $GABA_A$ receptors on VTA GABAergic neurons (*15*) are involved in the change in opiate reward system after intra-VTA BDNF infusion, we explored the effects of intra-VTA infusion of the $GABA_A$ receptor antagonist bicuculline. In drug-nondependent rats, intra-VTA bicuculline (50 ng) produced a robust rewarding effect through a non-dopaminergic, TPP-dependent reward system ($P < 0.05$). However, in drug non-dependent rats infused with intra-VTA BDNF (n = 11) [but not PBS (n = 8)], alpha-flupenthixol now blocked the rewarding effects of bicuculline ($P > 0.05$) ($F_{1,37}$ = 5.88, $P < 0.05$, interaction of BDNF and bicuculline). (Fig. 2A). To study the physiological effects of BDNF on $GABA_A$ inhibition of VTA GABA neurons, we evaluated the response of VTA GABA neurons to in situ administration of the $GABA_A$ agonist muscimol. Single-unit extracellular recordings of VTA GABAergic neurons (*16*) (four to five cells from rat) revealed that a shift from inhibitory to excitatory $GABA_A$ receptor signaling was observed in a subset of GABAergic neurons. Iontophoretic application of muscimol excited 42% [12 out of 31 (12/31)] of VTA GABAergic neurons in intra-VTA BDNF-infused rats (n = 8) compared with intra-VTA PBS (n = 8) infused controls, where 100% (29/29) of the VTA GABAergic neurons were inhibited by muscimol (Mann-Whitney U test, $P < 0.05$) (Fig. 2, B and C). This shift is very similar to that seen in a previous study with opiate-dependent rats, where 44% of VTA GABAergic neurons switched to excitatory $GABA_A$ receptor signaling (*2*). In BDNF-treated rats (16.6 ± 8.9%, n = 11), as in control PBS-treated rats (19.5 ± 9.26%, n = 7), the $GABA_B$ receptor agonist baclofen was still able to inhibit those GABA neurons that had been excited by muscimol (t_{17} = 0.216, $P > 0.05$) (Fig. 2, B and C), suggesting very specific effects of BDNF on $GABA_A$ receptor signaling on the VTA GABAergic neurons themselves.

The intra-VTA alterations that lead to this transformation are unclear. BDNF may change the anion gradient by reducing the level of the potassium-chloride co-transporter KCC2, so that the GABAergic neuron intracellular chloride concentration increases (*6*, *17*). $GABA_A$ receptor activation would then result in anions flooding out of the neuron, making the neuron's membrane potential more positive, or depolarized, relative to the resting membrane potential. Also, BDNF might elevate intracellular carbonic anhydrase levels, favoring HCO_3^- efflux and resulting in a depolarizing response in VTA GABAergic neurons (fig. S1) (*2*).

The present work suggests that BDNF in the VTA induces a transition to a drug-dependent motivational state, a crucial issue in drug addiction research. Other studies have found that BDNF levels within the mesolimbic system progressively increase after cocaine withdrawal (*18*). Intra-VTA BDNF infusion produces long-lasting enhancement of cocaine seeking (*18*) and locomotor stimulation (*6*, *9*), and it increases the rewarding effects of cocaine itself (*9*). Conversely, cocaine-conditioned place preference was reduced in heterozygous BDNF knockout mice (*9*), and inhibiting BDNF activity reduces amphetamine-induced dopamine release (*19*). Our findings complement research in animal and clinical studies suggesting that VTA BDNF is implicated in the pathogenesis of drug addiction (*20*).

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