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Neurotrophin-4 is required for tolerance to morphine in the mouse

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Abstract

Tolerance is an important component of opiate addiction, but the molecular basis for this phenomenon remains obscure. Here, we report that mice lacking neurotrophin-4 (NT4) display substantially reduced tolerance to morphine compared to wild-type. However, there were no deficits in sensitization and withdrawal, other behaviors relevant to drug addiction. Since NT4 knockout mice also show abnormalities in long-term but not short-term memory, our findings suggest common molecular pathways for some of the enduring changes of drug addiction and memory consolidation.

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Drug addiction results in complex behavioral changes including dependence, tolerance, sensitization and craving. Some of these behaviors can persist for a lifetime [5,10,14]. The molecular basis of addiction remains poorly understood, but based on expression studies some of the relevant genes appear to be shared with those for learning and memory, suggesting a molecular connection between these apparently unrelated behaviors. From a neuroanatomical perspective, the locus coeruleus, nucleus accumbens, ventral tegmental area and periaqueductal gray all seem to play roles in addiction. Less well understood is the role of other areas of the brain, such as the cerebral cortex, hippocampus, and limbic system.

The neurotrophins include nerve growth factor, brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT3) and neurotrophin-4 (NT4) [1]. Diverse lines of evidence suggest that these growth factors play a role in regulation of synaptic plasticity. Two types of receptors mediate the functions of the neurotrophins, the trks and p75. The four neurotrophins are ligands for p75, a receptor that belongs to tumor necrosis factor receptor superfamily. The main receptor for BDNF and NT4 is trkB, a member of the trk family of receptor tyrosine kinases.

Knockouts of the NT4 gene have been created in mice [3,

8]. These mice represent the only strain of neurotrophin knockouts that are homozygous viable. The NT4 knockouts lack any overt phenotype, with the exception of neuronal reductions in the nodose and geniculate ganglia. These peripheral ganglia represent the location of the visceral and facial sensory neuronal cell bodies. Behaviorally however, the NT4 knockout mice are defective in long-term but not short-term memory [16]. In addition, abnormalities are present in long-lasting hippocampal long-term potentiation (LTP) but not decremental LTP. These findings suggested that NT4 plays an important role in consolidation of long-term memory and also long-lasting LTP.

Here we present an examination of the role of NT4 in addiction. Strikingly, mice lacking NT4 showed decreases in morphine tolerance, but not sensitization or withdrawal. The memory and LTP deficits of the NT4 knockout mice on the one hand, and the alterations in opiate tolerance on the other, is evidence in favor of common molecular pathways for these long-lasting behaviors.

The wild-type and NT4 knockout mice were on the 129X1/SvJ background [16]. Tolerance was tested using a hot-plate maintained at 55 °C. The behavioral endpoint was lifting of one of the hindpaws, licking of the forepaws or jumping. In the absence of a response within 60 s, the cut-off value, the mice were removed from the hot-plate to prevent burns. On the first day each mouse was placed on the hot-plate and the latency to endpoint noted. Mice were then

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injected with 3 mg kg⁻¹ morphine sulfate i.p. and retested on the hot-plate 15 min after injection. The next day mice were given 9 mg kg⁻¹ morphine sulfate i.p. and again tested. The percentage antinociception was calculated for each animal as [(post-drug latency) – (baseline latency)] / [(cut-off value) – (baseline latency)]. Chronic morphine treatment consisted of 4 days of morphine, 5 mg kg⁻¹ i.p. twice per day [4]. On the 5th day, the animals were treated with morphine in the morning and challenged 9 h later with 3 mg kg⁻¹ morphine and tested on the hot-plate. The following day the mice were challenged with 9 mg kg⁻¹ morphine and again tested.

Sensitization was assessed [6,15] by quantitating locomotor activity using the Cage Rack System (San Diego Instruments) [12,13]. This system uses a uniformly spaced 8 × 4 photobeam grid to monitor activity in cages 47 (long) × 26 (short) × 15 cm (high). Mice were provided with food and water and monitored in the dark after injection with morphine. Locomotor activity was measured by counting the total number of beam breaks during a 1 h testing period. The following schedule was employed: on day 0, untreated mice were assessed; on day 1 mice injected with vehicle; and on days 2–6 and day 14, mice injected with 10 mg kg⁻¹ i.p. morphine sulfate.

Withdrawal was tested by first inducing physical dependence upon morphine using the following regimen. On the first day, mice were injected with three doses of morphine sulfate, 20, 40, 60 mg kg⁻¹ i.p. spaced by 4 h intervals. On the second day, the mice were injected with 80, 100, 100 mg kg⁻¹ i.p. On the last day, the mice were injected with 100 mg kg⁻¹ i.p. and two h later given a 1 mg kg⁻¹ dose of naloxone to precipitate withdrawal. Mice were scored for 30 min after naloxone injection for wet dog shakes, jumps and paw tremors [9].

To assess the effect of NT4 on morphine tolerance, both wild-type and knockout mice were challenged with morphine either after chronic morphine treatment or in its absence. The hot-plate assay was used to assess the antinociceptive effects of the morphine challenge. This assay quantitates the relative increase in latency of withdrawal from a hot-plate caused by analgesic administration. An acute dose of 3 mg kg⁻¹ of morphine resulted in significant antinociception for both wild-type and knockout mice, with no differences resulting from genotype (Fig. 1). After 5 days of chronic morphine administration, the same acute dose of 3 mg kg⁻¹ no longer had an antinociceptive effect on the wild-type controls, indicating successful induction of tolerance. In contrast, after chronic morphine administration, the NT4 knockout mice showed a significantly different antinociceptive response compared to wild-type. The knockout mice still showed significant antinociception in response to the acute dose of morphine, indicating that tolerance had not been induced. Similar results were found using a higher acute dose of 9 mg kg⁻¹ morphine (Fig. 1).

In order to assess whether the changes in morphine

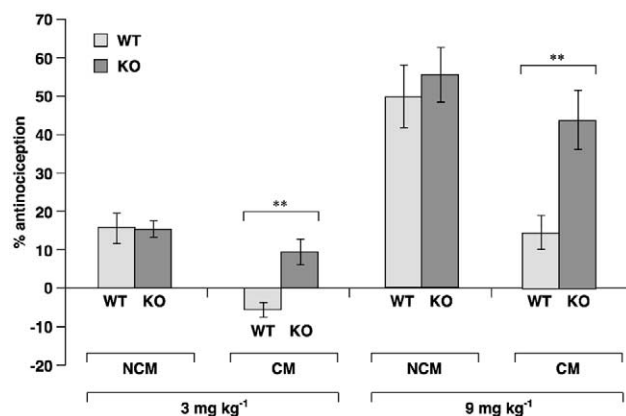


Fig. 1. Tolerance in wild-type and NT4 knockout mice. Mice were challenged with either 3 mg kg⁻¹ or 9 mg kg⁻¹ morphine, after no chronic morphine (NCM) or after chronic morphine (CM). After the challenge, antinociception was assessed using the hot-plate test. In the absence of chronic morphine, both wild-type ($P = 0.0016$, 3 mg kg⁻¹ challenge; $P < 0.0001$, 9 mg kg⁻¹ challenge) and knockout mice ($P < 0.0001$, 3 mg kg⁻¹ challenge; $P < 0.0001$, 9 mg kg⁻¹ challenge) showed significant antinociception. However, after chronic morphine, there was a significant decrease in antinociception for the wild-type ($P < 0.0001$, 3 mg kg⁻¹ challenge; $P = 0.0013$, 9 mg kg⁻¹ challenge), but not the knockout mice ($P = 0.16$, 3 mg kg⁻¹ challenge; $P = 0.25$, 9 mg kg⁻¹ challenge). In addition, the wild-type and knockout mice showed significantly different antinociception after chronic morphine ($P = 0.0016$, 3 mg kg⁻¹ morphine challenge; $P = 0.0067$, 9 mg kg⁻¹ morphine challenge), but not in the absence of chronic morphine ($P = 0.95$, 3 mg kg⁻¹ morphine challenge; $P = 0.58$, 9 mg kg⁻¹ morphine challenge). $n = 16$ for both wild-type and knockout mice. Mean \pm SEM is shown. ** $P < 0.01$, two-tailed t -test.

tolerance in the NT4 knockout mice were specific, or related to general changes in the behavioral responses to opiates, sensitization was quantitated. Mice were injected over successive days with morphine and the locomotor activity in a 1 h time period measured after each injection. Sensitization is manifested by a positive relationship between the locomotor activity and the number of times the mice are injected with morphine. In the 129X1/SvJ background employed for these studies, there was no significant main effect of the number of morphine injections on distance traveled (Fig. 2A). In addition, there was no effect of genotype. However, when the data was collapsed into non-treated and treated groups (Fig. 2B), a significant effect of the morphine treatment was now found, but again there was no effect of genotype. These data indicate that lack of NT4 did not change the sensitization to morphine, although this behavior was not prominent in the background strain employed.

Withdrawal was tested by subjecting the mice to chronic morphine treatment for 5 days using the same protocol employed for induction of tolerance. The response of the mice to naloxone precipitated morphine withdrawal was then assessed (Fig. 3). The total scores for both wild-type and control mice were significantly different from zero ($P < 0.05$, one-tailed t -test), suggesting successful induction of withdrawal. However, there were no significant

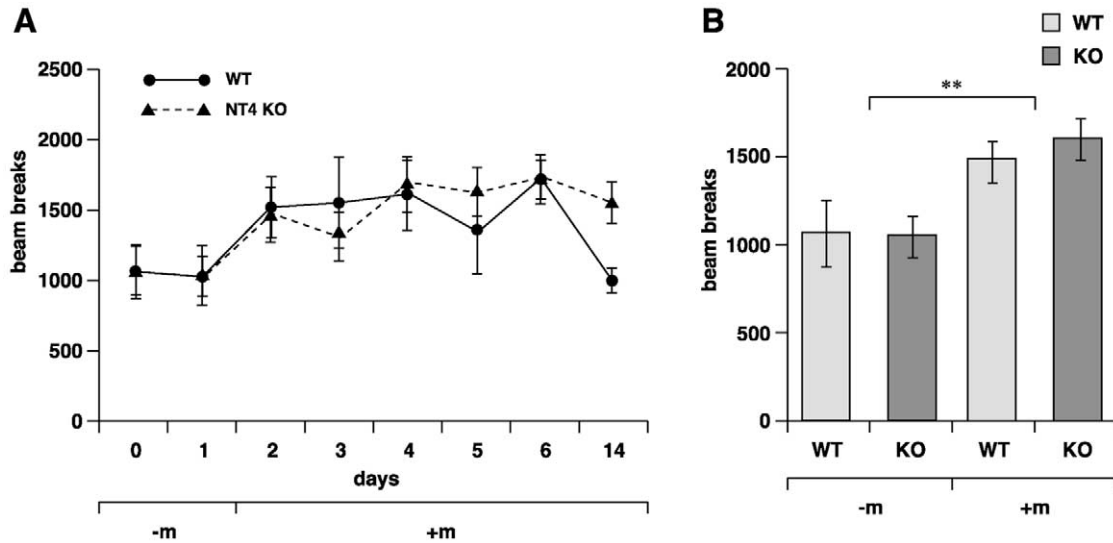


Fig. 2. Sensitization. (A) Day 0 represents untreated mice; day 1, mice injected with vehicle; days 2–6 and 14, mice injected with morphine. $n = 12$ for both wild-type and knockout mice. ANOVA showed no significant effect of either genotype ($P = 0.27$) or day of treatment ($P = 0.09$). – m, no morphine; + m, morphine treated. (B) The data in (A) is replotted by pooling data from non-morphine exposed mice (– m, days 0 and 1) and data from morphine exposed mice (+ m, days 2–6 and 14). There is a significant effect of morphine treatment ($P = 0.002$), but not genotype ($P = 0.56$).

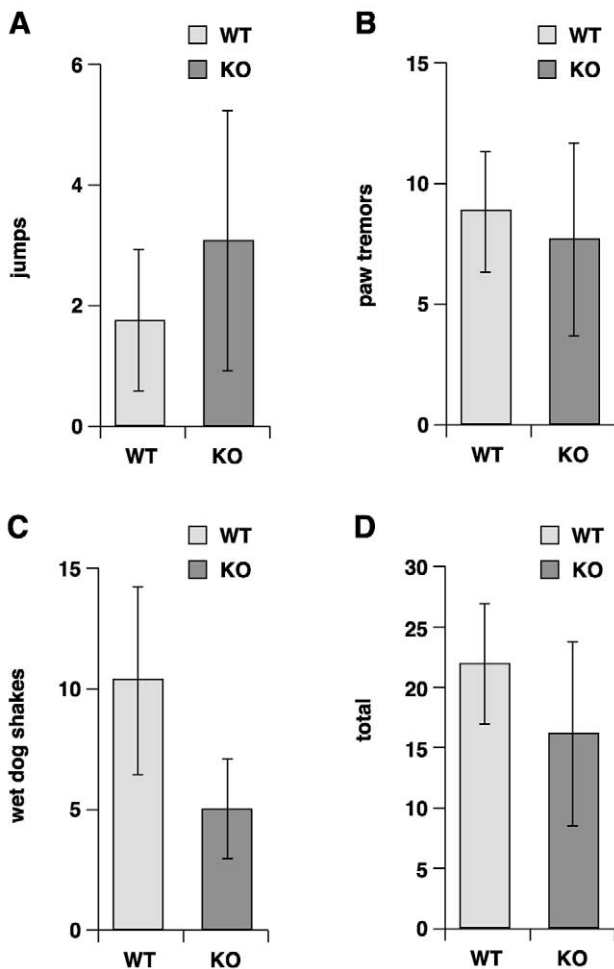


Fig. 3. Withdrawal. There was no significant effect of genotype for (A) jumps, (B) paw tremors, (C) wet dog shakes, or (D) total withdrawal score. $n = 11$, wild-type mice; $n = 12$, knockout mice.

differences between the wild-type and knockout mice for any of the withdrawal behaviors quantitated.

The enduring behavioral changes of addiction and memory has suggested that similar molecular mechanisms may be responsible for both. This conjecture is now supported by the present study, where it is shown that loss of the NT4 gene results in deficits in one long-lasting aspect of opiate addiction, namely tolerance. Since mice lacking NT4 also show abnormal long-term memory and long-lasting LTP [16], these observations suggest that NT4 is required for the synaptic plasticity mediating both addiction and memory. It is unlikely that the changes in tolerance in the NT4 knockout mice are due to deficits in nociception, since both the wild-type and control mice show the same sensitivity to electrical foot shock [16]. In addition, similar degrees of antinociception were shown by both groups of mice in response to acute morphine treatment in the absence of chronic morphine (Fig. 1).

The changes in the NT4 knockout mice were restricted to only one of the tested aspects of addiction, namely tolerance. No alterations were found in sensitization or withdrawal. This may mean that different molecular pathways are responsible for these aspects of addiction. However, any role of NT4 in sensitization and withdrawal might be more prominently displayed in backgrounds other than 129X1/SvJ. In contrast to the NT4 knockout mice, it has been shown that both mice with a conditional deletion of BDNF in postnatal brain and mice with a CREB gene knockout have normal tolerance but decreased withdrawal [2,9]. Perhaps CREB and BDNF are involved in related molecular pathways in the long-term changes of drug addiction, while NT4 plays a complementary role.

Although the NT4 knockout mice showed abnormalities in both memory and tolerance, NT4 may be acting in

different neuroanatomical regions in these behaviors. This issue could be tackled using tissue specific knockouts [7]. For learning and memory, NT4 may exert its effects through the hippocampus. Consistent with this notion is the alteration of long-term LTP in the NT4 knockouts. For morphine tolerance, NT4 may be acting in regions relevant to addiction, such as the nucleus accumbens and locus coeruleus. Interestingly, BDNF and NT3, as well as their receptors *trkB* and *trkC*, show expression changes in the locus coeruleus as a result of both morphine withdrawal and chronic administration [11].

Also pertinent is whether absence of NT4 blocks tolerance only if the deficiency occurs during the entire period of drug exposure, or whether absence of NT4 can block tolerance even after induction of this behavior. This point could be addressed using temporal specific knockouts [17]. Tolerance is a significant component of drug addiction, leading to ever increasing use and risk of overdose. If absence of NT4 can block tolerance even after its initiation, antagonism of the NT4 pathway could have important therapeutic implications.

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