Opiate anticipation, opiate induced anatomical changes in hypocretin (Hcrt, orexin) neurons and opiate induced microglial activation are blocked by the dual Hcrt receptor antagonist suvorexant, while opiate analgesia is maintained

Ronald McGregor,^{*}1 Ming-Fung Wu,^{*1} Thomas C. Thannickal,^{*1} and <u>Jerome M. Siegel^{#,1}</u>

Author information Copyright and License information PMC Disclaimer

The complete version history of this preprint is available at bioRxiv.

Go to:

Abstract

 in the number of detected Hcrt neurons and a decrease in their soma size. We now show that the increased number of Hcrt cells visible after morphine treatment is likely the result of increased Hcrt production in neurons ha show that the increased number of Hcrt cells visible after morphine treatment is likely the result of increased Hcrt production in neurons having sub-detection level of the peptides. We find that morphine increases Hcrt pr likely the result of increased Hcrt production in neurons having sub-detection levent of the peptides. We find that morphine increases Hcrt projections to the ventral tegmental area (VTA), the level of tyrosine hydroxylase of the peptides. We find that morphine increases Hcrt projections to the ventral
tegmental area (VTA), the level of tyrosine hydroxylase enzyme (TH) and the
number of TH positive cells in VTA, with no changes in the adjace tegmental area (VTA), the level of tyrosine hydroxylase enzyme (TH) and the number of TH positive cells in VTA, with no changes in the adjacent substantia n We find that the dual Hcrt receptor antagonist suvorexant prevent number of TH positive cells in VTA, with no changes in the adjacent substantiated We find that the dual Hcrt receptor antagonist suvorexant prevents morphine induced changes in the number and size of Hcrt neurons, microgli

We find that the dual Hcrt receptor antagonist suvorexant prevents morphine-
induced changes in the number and size of Hcrt neurons, microglial activation and
morphine anticipatory behavior, but does not diminish morphine disease, bone damage and many other painful conditions often cannot be effectively morphine anticipatory behavior, but does not diminish morphine analgesia. These findings suggest that combined administration of opiates and suvorexant may be a less addictive way of administering opiates for pain relief i findings suggest that combined administration of opiates and suvorexant may be a
less addictive way of administering opiates for pain relief in humans.
The annual US rate of opioid overdose deaths now exceeds 76,000, much less addictive way of administering opiates for pain relief in humans.
The annual US rate of opioid overdose deaths now exceeds 76,000, much greater
than the annual rates for automobile or gun deaths (CDC website). This i The annual US rate of opioid overdose deaths now exceeds 76,000, m
than the annual rates for automobile or gun deaths (CDC website). Th
contrast to the annual US opioid overdose death rate of 8,000 records
Of those who beg than the annual rates for automobile or gun deaths (CDC website). This is in contrast to the annual US opioid overdose death rate of 8,000 recorded before 19 Of those who began abusing opioids in the 2000s, 75 percent rep contrast to the annual US opioid overdose death rate of 8,000 recorded beform of those who began abusing opioids in the 2000s, 75 percent reported that that first opioid was prescribed for the relief of pain, α . This pr Of those who began abusing opioids in the 2000s, 75 percent reported that their first opioid was prescribed for the relief of pain, α . This progressed to illegal opioid pill acquisition or to heroin or fentanyl use- α first opioid was prescribed for the relief of pain, $\frac{1}{4}$. This progressed to illegal opioipill acquisition or to heroin or fentanyl use $\frac{1}{4}$. Although non-opioid analgesics can used for relatively minor pain, sev first opioid was prescribed for the relief of pain, 2. This progressed to inegal opioid
pill acquisition or to heroin or fentanyl use--. Although non-opioid analgesics can b
used for relatively minor pain, severe burns, ca pin acquisition or to heroin or fentanyl usess. Although non-opioid analgesics can be
used for relatively minor pain, severe burns, cancer, joint inflammation, sickle cell
disease, bone damage and many other painful condit disease, bone damage and many other painful conditions often cannot be effectivel
treated with non-opioid analgesics. These disorders cause immense suffering.
We and others have demonstrated that increased neuronal dischar

disease, both non-opioid analgesics. These disorders cause immense suffering.
Wesselland other that have demonstrated that increased neuronal discharge in
hypocretin (Hcrt, orexin) neurons is linked to the performance of r treated with the separation of the contracted with the contracted with the sum of the Network Western (Hert, origin) neurons is linked to the performance of rewarded tax approach (Hert, origin) neurons is linked to the per We-10 and others11 have demonstrated that increased neuronal discharge in
hypocretin (Hcrt, orexin) neurons is linked to the performance of rewarded
 hypocretin (Hcrt, orexin) neurons is linked to the performance of rewarded tasks in the performance of rewarded
In the performance of rewarded tasks in the performance of rewarded tasks in the performance of rewarded tasks

genetically knocked out (Hcrt-KOs) learn a bar press task for food or water
quickly as their WT littermates. However, when the effort to obtain the rev
increased in a "progressive ratio," they all quit the task within 1 h, quickly as their WT littermates. However, when the effort to obtain the reward increased in a "progressive ratio," they all quit the task within 1 h, whereas all WT littermates continue bar pressing until the end of the 2 increased in a "progressive ratio," they all quit the task within 1 h, whereas all the WT littermates continue bar pressing until the end of the 2 h test period. In contrathe Hcrt-KOs perform as well as WT controls on prog WT littermates continue bar pressing until the end of the 2 h test period. In contrast
the Hcrt-KOs perform as well as WT controls on progressive ratio avoidance tasks,
suggesting an emotional specificity in their response the Hcrt-KOs perform as well as WT controls on progressive ratio avoidance tasks, suggesting an emotional specificity in their response deficit. Normal dogs playing in a yard have a large increase in cerebrospinal fluid Hc suggesting an emotional specificity in their response deficit. Normal dogs playing is a yard have a large increase in cerebrospinal fluid Hcrt level. But when these same dogs are made to run on a treadmill, there is no cha

suggesting an emotional repeatively in their response that they have a large increase in cerebrospinal fluid Hcrt level. But when these same dogs are made to run on a treadmill, there is no change in Hcrt level, despite s dogs are made to run on a treadmill, there is no change in Hcrt level, despite simila
elevations of heart rate, respiratory rate and blood pressure». We found that Hcrt is
released in the brain of humans when they are enga elevations of heart rate, respiratory rate and blood pressures. We found that Hcrt is
released in the brain of humans when they are engaged in tasks they enjoy, but not
when they are aroused by pain or when they are feelin eleased in the brain of humans when they are engaged in tasks they enjoy, but not
released in the brain of humans when they are engaged in tasks they enjoy, but not
when they are aroused by pain or when they are feeling sa when they are aroused by pain or when they are feeling sad...
Dopamine neurons, particularly those located in the ventral tegmental area (VTA)
are known to play a significant role in reinforcement in general and in opiate When they are aroussed by pain or when they are feeling sadily
Dopamine neurons, particularly those located in the ventral to
are known to play a significant role in reinforcement in general
disorder (OUD) in particularly are known to play a significant role in reinforcement in general and in opiate use
disorder (OUD) in particularly all referred in the ventral tegemental and in opiate use
disorder (OUD) in particularly Hert and dopamine ar are known to play a significant role in reinforcement in general and in opiate use disorder (OOD) in particular¹³⁻¹⁵. Hcrt and dopamine are evolutionarily inked from
both a neurochemical and anatomical perspectives. VTA plasticity associated with
drug rewards requires functional Hcrt receptors. The lev

both a neurochemical states functional Hert receptors». The levels of dopamine and its
major metabolites in the nucleus accumbens are markedly increased by the
microinjection of Hert into the VTA®,®. Hert neurons project s drug contract the method is in the nucleus accumbens are markedly increased by the microinjection of Hcrt into the VTA_{^{n, 21}. Hcrt neurons project strongly to the nucleus accumbens and the paraventricular nucleus of the} microinjection of Hcrt into the VTA

accumbens and the paraventricular nucleus of the thalamus... Thus, Hcrt can

strongly modulate circuits implicated in OUD.

It has long been noted that human narcoleptics, who have an a merompection of Hcrt into the VTA1, 2. Hcrt neurons project strongly to the interests
accumbens and the paraventricular nucleus of the thalamus. Thus, Hcrt can
strongly modulate circuits implicated in OUD.
It has long been accumbens and the paraventricular indeeds of the thalamus20. Thus, Hcrt can strongly modulate circuits implicated in OUD.
It has long been noted that human narcoleptics, who have an average 90% lo
Hcrt neurons and very low It has long been noted that human narcoleptic
Hcrt neurons and very low CSF levels of Hcrt, s
abuse, dose escalation or overdose¹ despite th
hydroxybutyrate (GHB), methylphenidate and
reverse the sleepiness and cataplexy Hert neurons and very low CSF levels of Hert, show little if any evidence of drug
abuse, dose escalation or overdose¹ despite their daily prescribed use of gamma
hydroxybutyrate (GHB), methylphenidate and amphetamine. Th abuse, dose escalation or overdose_{^a} despite their daily prescribed use of gamma
hydroxybutyrate (GHB), methylphenidate and amphetamine. These drugs, which
reverse the sleepiness and cataplexy of narcolepsy, are frequen

abuse, dose estalation or overdose and any prediction. These drugs, which
they droxy butyrate (GHB), methylphenidate and amphetamine. These drugs, which
reverse the sleepiness and cataplexy of narcolepsy, are frequently a reverse the sleepiness and cataplexy of narcolepsy, are frequently abused in the general population with considerable loss of lifea-a, Human narcoleptics have also been shown to have a greatly reduced reward activation of general population with considerable loss of lifestate. Human narcoleptics have als
been shown to have a greatly reduced reward activation of the VTA, amygdala an
accumbens¹⁴ and altered processing of humor in the hypot general population with considerable loss of ineasa. Human narcoleptics have also been shown to have a greatly reduced reward activation of the VTA, amygdala and accumbens²² and altered processing of humor in the hypotha accumbens» and altered processing of humor in the hypothalamus and amygdala».
As we discovered, long term self-administration of heroin in humans or giving
addictive levels of morphine to mice, produces a substantial incre decalises. The aftered processing of hallof in the hypothalamus and amygdala-.
As we discovered, long term self-administration of heroin in humans or giving
addictive levels of morphine to mice, produces a substantial incr addictive levels of morphine to mice, produces a substantial increase in the nume of detected Hcrt neurons.² Cocaine or fentanyl produce similar changes in rats We also reported that heroin self-administration in humans of detected Hcrt neurons³⁸. Cocaine or fentanyl produce similar changes in rats²⁹, ³⁸. We also reported that heroin self-administration in humans and daily morphine injection in mice for 14 days, produce a marked sh of detected Hcrt neurons². Cocalite of fentanyl produce similar changes in rats2, 2.
We also reported that heroin self-administration in humans and daily morphine
injection in mice for 14 days, produce a marked shrinkag injection in mice for 14 days, produce a marked shrinkage of Hcrt neurons¹⁴. Becare of the role of Hcrt neurons in reward in rodents and pleasure in humans that we₄¹² and others¹² have seen, we wanted to rule out injection in infect of 14 days, produce a marked shrinkage of Hcrt neurons₂. Because
of the role of Hcrt neurons in reward in rodents and pleasure in humans that
we_s a and othersa have seen, we wanted to rule out the wes, 12 and others12 have seen, we wanted to rule out the possibility that blocking
that block in the possibility that blocking \mathbf{r} $we₅ = and other $5\pm$ have seen, we want to rule out the possibility that blocking their$

Go to:

Results

receptors might affect the target regions that mediate receptors may be exampled above.

Results

Mechanisms underlying the increased number of detected Hcrt neurons and is

shrinkage in Hcrt neuron size caused by opiate Results
Mechanisms underlying the increase
shrinkage in Hcrt neuron size cause Mechanisms underlying the increased number of detected Hcrt neurons and the shrinkage in Hcrt neuron size caused by opiates (Fig 1)

$Fig 1:$

Mechanisms underlying the increased number of detected Hcrt neurons and the shrinkage in Hcrt neuron size caused by opiates²⁸.
Human heroin addicts (*Fig* 1(a)), and mice given 50 mg/kg of morphine for a 14 or more days, showed an

Mechandical
Mechandical
Huma
increa
cell nu
preced
ICV ad
increa .
a o ii p =
ld increase in the number of Hcrt producing neurons and a decreased size of these neurons, with less
intense immunohistochemical staining["]. These effects were blocked in mice by the concurrent
administration of the opioid intense immunohistochemical staining²². These effects were blocked in mice by the concurrent
administration of the opioid receptor blocker naltrexone <u>Fig 1(b),(c</u>). We observed no difference in
cell number 1b (n=4/cond intense immunohistochemical staining⁻. These effects were blocked in mice by the concurrent
administration of the opioid receptor blocker naltrexone <u>Fig 1(b),(c</u>). We observed no differencell number 1b (n=4/condition, cell number 1b (n=4/condition, t=0.68, df= 6, P=0.53) or size 1c (t=0.21, df=6, P=0.9) when morphine was
preceded by naltrexone.
ICV administration of the microtubule transport blocker colchicine (in otherwise drug free m

preceded by naltrexone.
ICV administration of the microtubule transport blocker colchicine (in otherwise drug free mice)
increased the number of "detected" Hcrt cells in mice by 44% (Fig 1(d)). The Increase in cell number precedent
increased the number of
increased the number of Increased the number of "detected" Hcrt cells in mice by 44% (Fig 1(d)). The Increase in cell number increased the number of "detected" Hert cells in micro-by 44% (Fig.1(d)). The Increase in cell number after a
Increase in cells in cells in cells in cells in cells in cell number after a fig.

Hert cell number was not further increased by chronic morphine administration. (f) Colchicine did not
significantly alter the number of melanin concentrating hormone (MCH) neurons.
Our prior work in human heroin addicts (significantly alter the number of melanin concentrating hormone (MCH) neurons.
Our prior work in human heroin addicts (Fig 1(a)), and in mice given 50 mg/kg of
morphine for 14 or more days, showed that opiates increase th Our prior work in human heroin addicts (Fig 1(a)), and in mice given opphine for 14 or more days, showed that opiates increase the number of melaning producing neurons, decrease the size of these neurons, and reduction th morphine for 14 or more days, showed that opiates increase the number of Hert
producing neurons, decrease the size of these neurons, and reduce the intensity of
Hert neuron immunohistochemical staining.^a. Here we show t producing neurons, decrease the size of these neurons, and reduce the intensity
Hert neuron immunohistochemical staining³. Here we show that these effects are
blocked in mice given 50 mg/kg of the opioid antagonist nalt Furt neuron immunohistochemical staining_®. Here we show that these effects are blocked in mice given 50 mg/kg of the opioid antagonist naltrexone 30 min prior teach daily 50 mg/kg morphine dose for 14 days (Fig 1(b),(c) Hocked in mice given 50 mg/kg of the opioid antagonist naltrexone 30 min prior teach daily 50 mg/kg morphine dose for 14 days (<u>Fig 1(b), c</u>)). When naltrexone we given before morphine there were no significant changes in each daily 50 mg/kg morphine dose for 14 days (F<u>ig 1(b) (c)</u>). When naltrexone was
given before morphine there were no significant changes in Hcrt cell number (b)
(t=0.68, df=6, P=0.53) or size (c) (t=0.21, df=6, P=0.9) given before morphine there were no significant changes in Hcrt cell number (b)
(t=0.68, df=6, P=0.53) or size (c) (t=0.21, df=6, P=0.9) (n=4 per condition). **This**
shows that the morphine effects on Hcrt neurons size and shows that the morphine effects on Hcrt neurons size and number are mediated by opiate receptors. But naltrexone not only prevents opiate induced

 $g(t=0.68, df=6, P=0.53)$ or size (c) $(t=0.21, df=6, P=0.9)$ (n=4 per condition). This
shows that the morphine effects on Hcrt neurons size and number are
mediated by opiate receptors. But naltrexone not only prevents opiate ind (t=0.68, dr=6, r=0.53) or size (c) (t=0.21, dr=6, r=0.9) (n=+ per condition). This
shows that the morphine effects on Hcrt neurons size and number are
mediated by opiate receptors. But naltrexone not only prevents opiate mediated by opiate receptors. But nature above not only prevents opiate madecut changes in Hert neuron number and size and number, it also blocks opiate malgesia...
Colchicine, an inhibitor of microtubule polymerization, colchicine, an inhibitor of microtubule polymerization, prevents transport
peptides out of the cell soma. We found that intracerebroventricular injecticolchicine in naïve mice increases the number of Hcrt neurons by 44% <u></u> Colchicine,
peptides ou
colchicine i
which is col
for 14 days
produce it a
Tukey post
colchicine t
labelled, rel
ceiling to m
cells capabl peptides out of the cell soma. We found that intracerebroventricular injection colchicine in naïve mice increases the number of Hcrt neurons by $44\% \underline{\text{ Fig 1}}$ (which is comparable to the percent increase seen in mice afte colchicine in naïve mice increases the number of Hcrt neurons by 44% Fig 1(d), which is comparable to the percent increase seen in mice after morphine (50 mg for 14 days), i.e. as many as 44% of the neurons capable which is comparable to the percent increase seen in mice after morphine (50 mg
for 14 days), i.e. as many as 44% of the neurons capable of producing Hcrt do no
produce it at detectable levels under "baseline" conditions i for 14 days), i.e. as many as 44% of the neurons capable of producing Hcrt do not
produce it at detectable levels under "baseline" conditions in naïve mice (P<0.001,
Tukey post hoc, compared to control and saline conditio produce it at detectable levels under "baseline" conditions in naïve mice (P<0.001
Tukey post hoc, compared to control and saline conditions). <u>Fig 1(e</u>) shows that
colchicine together with morphine does not further incre Tukey post hoc, compared to control and saline conditions). Fig $1(e)$ shows that colchicine together with morphine does not further increase the number of cells labelled, relative to colchicine alone. Together, Fig $1(d)$ colchicine together with morphine does not further increase the number of cells
labelled, relative to colchicine alone. Together, <u>Fig 1(d)</u> & (e) show that there is a
ceiling to morphine effects on Hcrt neuronal number, labelled, relative to colchicine alone. Together, <u>Fig 1(d)</u> & <u>(e)</u> show that there is a
ceiling to morphine effects on Hcrt neuronal number, implying a fixed number of
cells capable of producing Hcrt, with 44% beyond th ceiling to morphine effects on Hcrt neuronal number, implying a fixed number of cells capable of producing Hcrt, with 44% beyond the control number of these celetected in mice and as much as 54% beyond the control number i cells capable of producing Hcrt, with 44% beyond the control number of these cel
detected in mice and as much as 54% beyond the control number in humans with
opiate use disorder.^{*a*}. **This is compatible with our conclusi** detected in mice and as much as 54% beyond the control number in humans with
opiate use disorder». This is compatible with our conclusion that the morphine
induced increase in Hcrt labelled neurons is not due to neurogenes opiate use disorder... This is compatible with our conclusion that the morphin
induced increase in Hcrt labelled neurons is not due to neurogenesis, but
rather due to accumulation of peptide in the cell somas... Fig 1(f) opiate use useful interacted in the disordered in the more conclusion that the morphine rather due to accumulation of peptide in the cell somas». Fig 1(f) shows that colchicine does not have any effect on the number of me induced increase in Hcrt labelled neurons is not due to neurogenesis, but rather due to accumulation of peptide in the cell somas. Fig $1(f)$ shows that rather due to accumulation of peptide in the cell somas. The right colochicine does not have any effect on the number of melanin concentrating hormone (MCH) neurons. MCH is a peptide of similar size to Hcrt. MCH neurons an

hormone (MCH) neurons. MCH is a peptide of similar size to Hcrt. MCH neur
intermingled with Hcrt neurons throughout the hypothalamus.
Suvorexant also blocks changes in Hcrt cell number and size produced by
morphine (Fig 2) intermingled with Hcrt neurons throughout the hypothalamus.
Suvorexant also blocks changes in Hcrt cell number and size produced by
morphine (Fig 2) Suvorexant also blocks changes in Hcrt cell number and size
morphine (Fig 2) Suvorexant also blocks changes in Hcrt cell number and size produced by morphine (Fig 2)

$Fig 2:$

Suvor
Cell nu
suvore
(P=0.4
Cell si
morp
from v
We r
daily
decre Subsequent blocked changes in interest: vehicle-morphine, morphine-suvorexant, and
suvorexant + morphine, all ***P=0.0001. Suvorexant alone did not significantly differ fre
f(P=0.482) in effect on cell number.
Cell size: F receptors with suvorexant, 30 mg/kg in 0.5% methylcellulose vehicle, by gavage, 60 (P=0.482) in effect on cell number.
Cell size: <u>Fig 2b</u>, Tukey difference test: vehicle vs morphine P=0.003, morphine vs suvorexant P=0.006,
morphine vs suvorexant + morphine **P=0.001. Suvorexant alone did not cause a si Cell size: Fig 2b, Tukey difference to
morphine vs suvorexant + morphin
from vehicle alone in effect on cell s
We reported that human her
daily for 14 days have a grea
decreased soma size of Hcrt
morphine (blue). Although morphine vs suvorexant + morphine **P=0.001. Suvorexant alone did not cause a significant difference
from vehicle alone in effect on cell size (P=0.985).
We reported that human heroin addicts-a and mice given 50 mg/kg of m from vehicle alone in effect on cell size (P=0.985).
We reported that human heroin addicts= and mice given 50 mg/kg of morphine
daily for 14 days have a greatly increased number of detected Hcrt neurons and
decreased soma We reported that human heroin addicts.
daily for 14 days have a greatly increase
decreased soma size of Hcrt neurons (Fi
morphine (**blue**). Although the dual Hcr
(**yellow**) had no significant effect on Hcr
receptors with s we reported that human mergin increased number of detected Hcrt neurons and decreased soma size of Hcrt neurons (Fig 1(a), Fig 2(a),2(b) vehicle (green) vs morphine (blue). Although the dual Hcrt receptor antagonist suvor decreased soma size of Hcrt neurons <u>(Fig 1(a), Fig 2(a),2(b)</u> vehicle (**green)** vs
morphine (**blue**). Although the dual Hcrt receptor antagonist suvorexant by itsel
(**yellow**) had no significant effect on Hcrt cell number decreased soma size of Hert held ons (Fig 1(a), Fig 2(a), 2(b) vehicle (green) vs
morphine (blue). Although the dual Hert receptor antagonist suvorexant by its
(yellow) had no significant effect on Hert cell number (a) or morphine (**blue**). Although the dual Hcrt receptor antagonist suvorexant by itself, (**yellow**) had no significant effect on Hcrt cell number (a) or size (b), blocking Hcrt receptors with suvorexant, 30 mg/kg in 0.5% methyl (yellow) had no significant effect on Hert cell number (a) or size (b), blocking Hert receptors with suvorexant, 30 mg/kg in 0.5% methylcellulose vehicle, by gavage, 60 min before each daily subcutaneous morphine (50 mg/k min before each daily subcutaneous morphine (50 mg/kg) injection, for 14 days in mice, completely prevented the opioid associated increase in the number (Fig 2a compare **blue** with **red**) elicited by morphine. The treatme mice, completely prevented the opioid associated increase in the number (Fig. 2a compare **blue** with **red**) elicited by morphine. The treatment effect on cell number, vehicle vs morphine, morphine vs suvorexant, and morphi $\frac{2a}{2a}$ compare **blue** with **red**) elicited by morphine. The treatment effect on cell
number, vehicle vs morphine, morphine vs suvorexant, and morphine vs suvor
number, vehicle vs morphine, morphine vs suvorexant, and $\frac{2a}{2a}$ compare blue with red) elicited by morphine. The treatment effect on cell
number, vehicle vs morphine, morphine vs suvorexant, and morphine vs suvo number, vehicle vs morphine, morphise, morphise, morphise, morphise, and more variable vs subsequent, and more
In the variable vari

not significantly differ from vehicle alone in effect on cell number (P=0.482).

Similarly, suvorexant prevented the reduction in Hcrt soma size produced by

morphine (Fig 2b): vehicle vs morphine P=0.003, morphine vs suv Similarly, suvorexant prevented the reduction in Hcrt soma size produced by
morphine (Fig 2b): vehicle vs morphine P =0.003, morphine vs suvorexant P-
morphine vs suvorexant + morphine P=0.001, Tukey post hoc test. Suvore morphine (<u>Fig 2b</u>): vehicle vs morphine P =0.003, morphine vs suvorexant P=
morphine vs suvorexant + morphine P=0.001, Tukey post hoc test. Suvorexand
did not significantly differ from vehicle alone in effect on cell siz morphine vs suvorexant + morphine P=0.001, Tukey post hoc test. Suvorexant alone
did not significantly differ from vehicle alone in effect on cell size
(P=0.985). **Therefore the Hcrt receptor blocker suvorexant prevents**

Suvorexant blocks morphine induced microglial activation in the hypothalamus and VTA (Fig 3)

did not significantly differ from vehicle alone in effect on cell size
(P=0.985). **Therefore the Hcrt receptor blocker suvorexant prevents**
morphine's effect on Hcrt neuron number and size.
Suvorexant blocks morphine in (P=0.985). **Therefore the Hcrt receptor blocker suvorexant pr**
 morphine's effect on Hcrt neuron number and size.

Suvorexant blocks morphine induced microglial activation in t

and VTA (Fig 3)

Fig 3:

Suvorexant block ($P = 0.985$). Therefore the Hcrt receptor blocker suvorexant prevents

Suvorexant blocks morphine induced microglial activation in the hypo

and VTA (Fig 3)

Fig 3:

Suvorexant blocked microglial activation in the hypotha mor pinne's effect on Hert incuron number and size.

Suvorexant blocks morphine induced microglial activ

and VTA (Fig 3)

Fig 3:

Suvorexant blocked microglial activation in the hypothalamus and VT

The number (a) and siz I sir i I i a l (I t si t i si si Fig 3:
Suvor
The nimorph
respection of Picture
in thy pot
treatm
saline
staine
staine The number (a) and size (b) of microglia in the hypothalamuc was signif
morphine (50 mg/kg) treatment (14 d) (n=5 per condition, P=0.028 and
respectively) compared to saline treated animals. Administration of suve
prior t morphine (50 mg/kg) treatment (14 d) (n=5 per condition, P=0.028 and P=0.020, Tukey post hoc,
respectively) compared to saline treated animals. Administration of suvorexant (30 mg/kg), 60 mi
prior to the morphine treatmen respectively) compared to saline treated animals. Administration of suvorexant (30 mg/kg) , 60 mi
prior to the morphine treatment completely blocked this effect on microglial number and size (P=0
and P=0.958, Tukey post h prior to the morphine treatment completely blocked this effect on microglial number and size (P=0.469 and P=0.958, Tukey post hoc, respectively, relative to saline) (c),(d),(e), show representative images of hypothalamic r and P=0.958, Tukey post hoc, respectively, relative to saline) (c),(d),(e), show representative images of hypothalamic sections stained for Iba-1 and cresyl violet solution from animals subjected to either saline (c), m hypothalamic sections stained for Iba-1 and cresyl violet solution from animals subjected to either salin(c), morphine (d) or suvorexant + morphine (e) illustrating microglial morphology. Similarly, in the VT_I region, th (c), morphine (d) or suvorexant + morphine (e) illustrating microglial morphology. Similarly, in the VTA region, the number (f) and size (g) of microglia was significantly increased by daily morphine (50 mg/kg) treatment region, the number (f) and size (g) of microglia was significantly increased by daily morphine (50 mg/kg) treatment (14d) (n=5 per condition, P=0.001 and P=0.001, Tukey post hoc, respectively) compared to saline treated a treatment (14d) (n=5 per condition, P=0.001 and P=0.001, Tukey post hoc, respectively) compared to
saline treatd animals. Administration of suvorexant (30 mg/kg), 60 minutes prior to morphine
treatment eliminated this eff saline treated animals. Administration of suvorexant (30 mg/kg) , 60 minutes prior to morphine treatment eliminated this effect in microglial number and size $(P=0.137$ and $P=0.09$, Tukey post hoc, respectively, relativ treatment eliminated this effect in microglial number and size $(P=0.137$ and $P=0.09$, Tukey post respectively, relative to saline). (h),(i),(j), show representative images of section containing the 1stained for Iba-1 and respectively, relative to saline). (h),(i),(j), show representative images of section containing the VTA stained for Iba-1 and cresyl violet solution from animals subjected to either saline (h), morphine (i) osuvorexant + σ stained for Iba-1 and cresyl violet solution from animals subjected to either saline (h), morphine (i) suvorexant + morphine (j). Inserts show the area outlined within the black square at higher magnification, illustr suvorexant + morphine (j). Inserts show the area outlined within the black square at higher magnification, illustrating microglial morphology. Scale bar 50µm, insert 10µm *P<0.05, ***P<0.001. magnification, illustrating microglial morphology. Scale bar $50 \mu m$, insert $10 \mu m * P < 0.05$, $***$ magnification, illustrating microglial morphology. Scale bar $50 \mu m$, insert $10 \mu m * P < 0.05$, $***$ magnification, illustrating microglial morphology. Scale bar 50μm, insert 10μm *P<0.05, ***P<0.001.

of **hypothalamic** Iba-1 labeled microglia and increased microglial soma size
compared to the saline condition (Fig 3(a),(b)) (P=0.028 and P=0.020,Tukey post
hoc, respectively). Suvorexant prevented the morphine induced ch

of hypothalamic Iba-1 labeled microglia and increased microglial soma size
compared to the saline condition (Fig 3(a),(b)) (P=0.028 and P=0.020,Tukey
hoc, respectively). Suvorexant prevented the morphine induced changes i hoc, respectively). Suvorexant prevented the morphine induced changes in microglia size and number (Fig 3(a),(b),(c),(d),(e)). There was no significant difference between microglial size and number in saline, vs suvorexan microglia size and number (Fig 3 (a),(b),(c),(d),(e)). There was no significant
difference between microglial size and number in saline, vs suvorexant plus
morphine treated mice (P = 0.469 and P=0.958).
Similarly, we obse difference between microglial size and number in saline, vs suvorexant plus
morphine treated mice (P = 0.469 and P=0.958).
Similarly, we observed that morphine treated mice had a significant increase
number and size of mi morphine treated mice (P = 0.469 and P=0.958).

Similarly, we observed that morphine treated mice had a significant increase

number and size of microglial cells in the **VTA** (<u>Fig 3(f),(g)</u>, P=0.001 for both

conditions, Similarly, we observed that morphine treated mi
number and size of microglial cells in the **VTA** (E
conditions, Tukey post hoc). Suvorexant adminis
treatment prevented these microglial changes in
There was no significant number and size of microglial cells in the **VTA** (Fig 3(f),(g), P=0.001 for both conditions, Tukey post hoc). Suvorexant administration prior to morphine treatment prevented these microglial changes in the VTA (Fig 3(f),(number and size of interogrameters in the VTA (Fig 3(f),(g), 1 –0.001 for both
conditions, Tukey post hoc). Suvorexant administration prior to morphine
treatment prevented these microglial changes in the VTA (Fig 3(f),(g) treatment prevented these microglial changes in the VTA. (Fig 3(f),(g),(h),(
There was no significant difference between number and size of microglia
comparing saline, and morphine + suvorexant in the VTA (P=0.137 and P=(There was no significant difference between number and size of microglial comparing saline, and morphine + suvorexant in the VTA (P=0.137 and P=0.09 respectively).
Effect of chronic morphine on Hcrt projections to, and ty

comparing saline, and morphine + suvorexant in the VTA (P=0.137 and P=1
respectively).
Effect of chronic morphine on Hcrt projections to, and tyrosine hydroxy
(TH) levels in, VTA and substantia nigra (Fig 4) respectively).
Effect of chronic morphine on Hcrt projections to, and tyrosine hydroxylase
(TH) levels in, VTA and substantia nigra (Fig 4)
P=0.099.099 Effect of chro
(TH) levels in Effect of chronic morphine on Hcrt projections to, and tyrosine hydroxylase (TH) levels in, VTA and substantia nigra $(Fig 4)$

Fig 4 :

Effect
Morpl
immu
condit
length
immu
test).
(P=0.0 Effect of chronic morphine on Hert projections to, and TH levels in, VTA and substantia nigra (SN).
Morphine treatment (once a day, 14d, 50 mg/kg, subcutaneous) resulted in a significant increase of Hcrt
immunofluorescenc immunofluorescence intensity per unit of area compared to saline treatment (a) in the VTA (n=4 per
condition for all measures, P=0.032, df = 6, t test). This was the result of a significant increase in the total
length of condition for all measures, $P=0.032$, df = 6, t test). This was the result of a significant increase in the t
length of Hcrt axons per unit area (b) (P=0.0072, df=6, t test). A significant elevation in TH
immunofluoresce length of Hcrt axons per unit area (b) (P=0.0072, df=6, t test). A significant elevation in TH
immunofluorescence intensity per unit of area compared to saline (c) was observed (P=0.021, df=6, t
test). This was accompanie immunofluorescence intensity per unit of area compared to saline (c) was observed (P=0.0
test). This was accompanied by a significant increase in the number of TH+ neurons in VTA
(P=0.0048, df=6, t test). Adjacent SN show in the st. This was accompanied by a significant increase in the number of TH+ neurons in VTA (d)
(P=0.0048, df=6, t test). Adjacent SN showed no difference in Hcrt (e) P=0.87 or TH (f), P=0.48, $(P=0.0048, df=6, t test)$. Adjacent SN showed no difference in Hcrt (e) P=0.87 or TH (f), P=0.48,
 $P=0.0048, df=6, t test$). Adjacent SN showed no difference in Hcrt (e) P=0.87 or TH (f), P=0.48, $(1 - 0.048, 0.048, 0.048)$ and $(1 - 0.048, 0.048)$ or TH (f), P=0.48, or TH (f), P=0.48,

morphine treatment. The difference in Hcrt innervation is visually apparent (h) between a saline treat
(top) and a morphine treated (bottom) animal. Inserts show higher magnification of the square areas i
yellow. Hcrt fib (top) and a morphine treated (bottom) animal. Inserts show higher magnification of the square areas in yellow. Hert fiber tracings of the VTA (green) (i) in a saline (top) or morphine (bottom) itracted animal. Representat (the tractions of the VTA (green) (i) in a saline (top) or morphine (bottom) treated animal.
Representative sections of the VTA (green) (i) in a saline (top) or morphine (bottom) illustrating the
difference in TH immunofl Representative sections of the VTA after saline (top) or morphine treatment (bottom) illustrating the difference in TH immunofluorescence (j). Scale bar 100 μ m; insert 20 μ m. ml = medial lemniscus. *P<0.01.

P<0.0 difference in TH immunofluorescence (j). Scale bar 100 μ m; insert 20 μ m. ml = medial lemniscus. *P<(P<0.01.)

Paily morphine (50 mg/kg) for 14 days increased Hcrt fluorescence intensity in the VTA Fig 4(a) (P=0.03 (a) 0.01.

Maily morphine (50 mg/kg) for 14 days increased Hcrt fluorescence intensity in the

UTA Fig 4(a) (P=0.032, n=4/condition, t test). The increase in intensity was

accompanied by a significant increase in Hcrt ax Daily mo
Daily mo
VTA <u>Fig</u>
accomp;
<u>4(b)</u>, P=
locus co
increase
previou;
with the
immunc
the num VTA Fig 4(a) (P=0.032, n=4/condition, t test). The increase in intensity was
accompanied by a significant increase in Hcrt axonal fiber density in the VTA Fig
4(b), P=0.0072, t test), comparable to what we have reported i accompanied by a significant increase in Hcrt axonal fiber density in the VT.
4(b), P=0.0072, t test), comparable to what we have reported in a prior students of all docus coeruleus (LC)_{³². We found that morphine treat} $4(b)$, P=0.0072, t test), comparable to what we have reported in a prior study in the locus coeruleus (LC)». We found that morphine treatment produces a significant increase in TH immunofluorescence in VTA (Fig 4c,,j),j), Hocus coeruleus (LC)[®]. We found that morphine treatment produces a significant
increase in TH immunofluorescence in VTA (<u>Fig 4c, j),</u> j), P=0.021, t test). We have
previously shown that TH immunofluorescence levels hav locus coerules in TH immunofluorescence in VTA (Fig 4c, j),j), P=0.021, t test). We have
previously shown that TH immunofluorescence levels have a positive correlation
previously shown that TH immunofluorescence levels ha

previously shown that TH immunofluorescence levels have a positive correlation
with the amount of TH protein present in the tissue». The increase in TH
immunofluorescence in this structure was accompanied by a significant with the amount of TH protein present in the tissue_¤. The increase in TH
immunofluorescence in this structure was accompanied by a significant increase
the number of TH+ neurons detected in morphine treated animals comp with the amount of TH protein present in the tissues." The increase in TH immunofluorescence in this structure was accompanied by a significant i
the number of TH+ neurons detected in morphine treated animals compa
contro the number of TH+ neurons detected in morphine treated animals compared to
controls (Fig 4(d), P=0.0048, t test).
Although the addiction related structures, LC and VTA, show increased Hcrt
innervation and TH immunofluores controls (Fig 4(d), P=0.0048, t test).
Although the addiction related structures, LC and VTA, show increased Hcrt
innervation and TH immunofluorescence, the motor related substantia nigra (St
adjacent to VTA had no signif Although the addiction related structures
innervation and TH immunofluorescondiacent to VTA had no significant chafter morphine (Fig 4e, P=0.87, t test)
P=0.48, t test) or TH+ cell number (F
4(h),(i),(i) show the differen innervation and TH immunofluorescence, the motor related substantia nigral
adjacent to VTA had no significant change in Hcrt immunofluorescence inter
after morphine (Fig 4e, P=0.87, t test), TH immunofluorescent intensity adjacent to VTA had no significant change in Hcrt immunofluorescence intensity
after morphine (Fig 4e, P=0.87, t test), TH immunofluorescent intensity (Fig 4f,
P=0.48, t test) or TH+ cell number (Fig 4g, P=0.32) after morp after morphine (Fig 4e, P=0.87, t test), TH immunofluorescent intensity (Fig 4f, P=0.48, t test) or TH+ cell number (Fig 4g, P=0.32) after morphine. Fig $4(h)$,(i),(i) show the difference in Hcrt innervation (green) and TH P=0.48, t test) or TH+ cell number (Fig 4g, P=0.32) after morphine. Fig
 $4(h)$,(i),(i) (i) show the difference in Hcrt innervation (green) and TH expression in
VTA (red) of a saline (top) vs a morphine treated animal (bot $\frac{4(h), (i), (j), (j)}{4(h), (j), (j)}$ show the difference in Hcrt innervation (green) and TH exploration (receptor blockade prevents conditioned morphine anticipation.
Hcrt receptor blockade prevents conditioned morphine anticipati VTA (red) of a saline (top) vs a morphine treated animal (bottom).
Hcrt receptor blockade prevents conditioned morphine anticipation (Fig.5)
 $\frac{5}{2}$

VERT FRAME CONTROLL INTERNATION OF A SALINO MORTAL CONTROLL OF A SALINO MORTAL CONTROLL OF A MORTAL CONTROLL OF A SALINO MUSIC SERVICE OF A MORTAL CONTROLL OF A MORTAL CONTROLL OF A MORTAL CONTROLL OF A MORTAL CONTROLL OF Hcrt receptor blockade prevents conditioned morphine anticipation (Fig 5)

available under [aCC-BY-NC-ND 4.0 International license.](http://creativecommons.org/licenses/by-nc-nd/4.0/) (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made bioRxiv preprint doi: [https://doi.org/10.1101/2023.09.22.559044;](https://doi.org/10.1101/2023.09.22.559044) this version posted February 17, 2024. The copyright holder for this preprint

Fig $5:$

Hert r
Fig 5a
group
morpl
Group
mg/kg
Antici
(blue
given
after r Here tect that the prevents conditioned morphine and pair and a second cohort of 3 groups of 6 mice given 10 mg/kg groups of 6 mice given 5 mg/kg of morphine, and a second cohort of 3 groups of 6 mice given 10 mg/kg morph From the figure of 6 mice given 5 mg/kg of morphine, and a second cohort of 3 groups of 6 mice given 10 mg/kg of
morphine (a total of 36 mice).
Groups were given vehicle (0.5% methylcellulose), used for suvorexant, with o morphine (a total of 36 mice).
Groups were given vehicle (0.5% methylcellulose), used for suvorexant, with or without suvorexant (30 mg/kg PO) at ZT4, followed by morphine or saline at ZT5. The 5 mg/kg group is shown in Fi Therefore is the Groups were given vehicle (0.5 mg/kg PO) at ZT4, followed by
Anticipatory running (**gray fill**)
(blue line) starting at ZT2 (2 b
given on the prior day). The areformation after morphine injection. The a mg/kg P0) at ZT4, followed by morphine or saline at ZT5. The 5 mg/kg group is shown in <u>Fig 5a</u>.
Anticipatory running (gray fill) is seen in the "vehicle at ZT4 then morphine at ZT5 group" under the
(blue line) starting at Anticipatory running (gray fill) is seen in the "vehicle at ZT4 then morphine at ZT5 group" under
(blue line) starting at ZT2 (2 hours after the light on pulse, and 21 hours after the last morphine
given on the prior day). Anticipatory running (gray fill) is seen in the "vehicle at 214 then morphine at 215 group" under the
(blue line) starting at ZT2 (2 hours after the light on pulse, and 21 hours after the last morphine dose
given on the pr (blue line) starting at ZT2 (2 hours after the light on pulse, and 21 hours after the last morphine dose,
given on the prior day). The anticipatory running continues to ZT5. Running further increased at ZT5
after morphine after morphine injection. The anticipatory running was absent in the group given suvorexant prior to after morphine injection. The anticipatory running was absent in the group given suvorexant prior to after morphine injection. The anticipatory running was absent in the group given surface $\frac{1}{2}$

morphine (ved met). The group given surorexant more of same (green line) also showed no
anticipatory running.
Fig 5b and andc<u>c</u> indicate total activity during two ZT (ZT2- ZT5 and ZT5-ZT10) interval, for each two morphine Fig 5b and andc<u>c</u> indict
Fig 5b and andcc indict
two morphine doses (
hoc test comparing to
Nertical yellow ba
studied 3 groups
first group **(blue**
mg/kg of morphii
vehicle (0.5% me
The third group w
at ZT4 and saline Figure 10.5% and 10 mg/kg (c)), used, *P<0.05, **P<0.01, *** P<0.001, Tukey post
hoc test comparing to vehicle + morphine condition.
Vertical yellow bars indicate light periods in skeleton light-dark cycle. Fig 5a: We
stu the test comparing to vehicle + morphine condition.

Vertical yellow bars indicate light periods in skeleton light-dark cycle. Fig 5a: We

studied 3 groups of 6 mice. Drugs were given at ZT(Zeitgeber Time) 4 and ZT5. The
 Vertical yellow bars indicate light periods
studied 3 groups of 6 mice. Drugs were giv
first group **(blue (V+M)** was given vehicle
mg/kg of morphine at ZT5. The second gro
vehicle (0.5% methylcellulose) at ZT4 and
The thir studied 3 groups of 6 mice. Drugs were given at ZT(Zeitgeber Time) 4 and ZT5. The first group (blue (V+M) was given vehicle (0.5% methylcellulose) PO at ZT4 and mg/kg of morphine at ZT5. The second group was given suvorex

first group (**blue (V+M)** was given vehicle (0.5% methylcellulose) PO at ZT4 and 5 mg/kg of morphine at ZT5. The second group was given suvorexant 30 mg/kg in vehicle (0.5% methylcellulose) at ZT4 and 5 mg/kg of morphine first group (blue (V+M) was given ventice (0.5% methylcellulose) 1 0 at 214 and 3 mg/kg of morphine at ZT5. The second group was given suvorexant 30 mg/kg of morphine at ZT5 (red (S+M) The third group was given suvorexant wehicle (0.5% methylcellulose) at ZT4 and 5 mg/kg of morphine at ZT5 (red (S+N)
The third group was given suvorexant 30 mg/kg in vehicle (0.5% methylcellulose
at ZT4 and saline at ZT5 (green (S+S).
Fig 5a shows wheel runn vehicle (0.5% methylcellulose) at ZH + and 5 mg/kg of morphine at ZH5 (red (S+M).
The third group was given suvorexant 30 mg/kg in vehicle (0.5% methylcellulose)
at ZT4 and saline at ZT5 (green (S+S).
Eig 5a shows wheel ru at ZT4 and saline at ZT5 **(green (S+S)**.

Fig 5a shows wheel running averaged over the last 12 days of the 14 day study

periods for the three morphine 5 mg/kg groups: Anticipatory running **(gray fill)** is

seen in the "v Fig 5a shows wheel running averaged c
Fig 5a shows wheel running averaged c
periods for the three morphine 5 mg/k
seen in the "vehicle at ZT4 then morphi
at ZT2 (2 hours after the light on pulse
injection) and continuing t periods for the three morphine 5 mg/kg groups: Anticipatory running (**gray fil**
seen in the "vehicle at ZT4 then morphine at ZT5 group" (**blue (V+M)** line, start
at ZT2 (2 hours after the light on pulse and 3 hours **befor** periods for the three morphine and graphs. Anticipatory running (gray min) is seen in the "vehicle at ZT4 then morphine at ZT5 group" (blue (V+M) line, starting in at ZT2 (2 hours after the light on pulse and 3 hours **bef** seen in the ventre at z_{T4} then interparate at z_{T4} starting interparate at zT4 group" (blue (V+M) line, starting
injection) and continuing to ZT5. Anticipatory wheel running is starting by day 3
presumably as a result o at 212 (2 hours after the light on pulse and 3 hours **before** the daily lhol plinte injection) and continuing to ZT5. Anticipatory wheel running is starting by day presumably as a result of anticipation conditioned by the injection) and continuing to ZT5. Anticipatory wheel running is starting by day 3 injections at ZT 5, 21 hours earliers at This anticipation was **absent** in the suvorexant + morphine group (**red (S+M**), which received suvorexant 1 hour b the morphine dose 21 hours earlier. Running in both groups furthe injections at ZT 5, 21 hours earlier³²². This anticipation was **absent** in the
suvorexant + morphine group (**red (S+M**), which received suvorexant 1 h
the morphine dose 21 hours earlier. Running in both groups further in suvorexant + morphine group (red (S+M), which received suvorexant 1 hour before the morphine dose 21 hours earlier. Running in both groups further increased at ZT5 after morphine injection. There was also a marked increase ZT5 after morphine injection. There was also a marked increase in running in bot
morphine groups' activity after the mice were handled at ZT4 for vehicle or
suvorexant administration. Suvorexant greatly reduced running af morphine groups' activity after the mice were handled at ZT4 for vehicle or
suvorexant administration. Suvorexant greatly reduced running after morphine
injection at ZT5-ZT8 compared to the vehicle-then morphine group, in suvorexant administration. Suvorexant greatly reduced running after morplinjection at ZT5-ZT8 compared to the vehicle-then morphine group, indication and on morinduced motor excitation. The **(green (S+S)** line shows the l

injection at ZT5-ZT8 compared to the vehicle-then morphine group, indicating a
major dampening by suvorexant on both morphine anticipation and on morphin
induced motor excitation. The (**green (S+S**) line shows the lack of major dampening by suvorexant on both morphine anticipation and on morphin
induced motor excitation. The (**green (S+S**) line shows the lack of activity in the
suvorexant and saline group, which experienced the same handli induced motor excitation. The (**green (S+S)** line shows the lack of activity in the suvorexant and saline group, which experienced the same handling as the other groups but was not given morphine.
We ran these conditions mateca motor excitation. The (green (S+S) line shows the lack of activity in the suvorexant and saline group, which experienced the same handling as the other groups but was not given morphine.
We ran these conditions wit groups but was not given morphine.
We ran these conditions with both 5 and 10 mg/kg doses of morphine with a
virtually identical pattern of activity in both experiments (the 5 mg dose is show
in Fig 5(a)). Bar graphs (Fig We ran these conditions with both 5
virtually identical pattern of activity
in Fig 5(a)). Bar graphs (Fig 5(b) & F
intervals, for each of the two morphi
morphine condition, vehicle vs morp
suvorexant + morphine (P<0.05; P wirtually identical pattern of activity in both experiments (the 5 mg dose is sh
in <u>Fig 5(a)</u>). Bar graphs (Fig 5(b) & Fig 5(c)) indicate total activity during two
intervals, for each of the two morphine doses used. Comp in <u>Fig 5(a)</u>). Bar graphs (<u>Fig 5(b)</u> & <u>Fig 5(c)</u>) indicate total activity during two ZT intervals, for each of the two morphine doses used. Comparisons to vehicle-
morphine condition, vehicle vs morphine, morphine vs s intervals, for each of the two morphine doses used. Comparisons to vehicle-
morphine condition, vehicle vs morphine, morphine vs suvorexant, and morphine
suvorexant + morphine (P<0.05; P<0.01, P<0.001,Tukey post hoc test, suvorexant + morphine (P<0.05; P<0.01, P<0.001,Tukey post hoc test, respectively).
Effect of suvorexant on threshold (Fig 6)

Effect of suvorexant on threshold $(Fig 6)$
The contract of suvorexant on threshold $(Fig 6)$ Effect of suvorexant on threshold (Fig 6)

Fig 6 :

Effect
The are baseli
elevat
elevat
mg/kg
both 5
vehicl
analge
suvore Encet of suvorexant on pain.
The analgesic effect of morphin
baseline (vehicle alone) to 5 mg
elevation of nociceptive thresh
mg/kg oral (by gavage) dose of
both 5 mg/kg or 10 mg/kg mor
vehicle and suvorexant + morp
analge baseline (vehicle alone) to $\frac{1}{5}$ mg/kg or 10 mg/kg, morphine doses. The average analgesic effect (i.e.
elevation of nociceptive threshold to heat) (n=6/group, 3 tests) was not significantly diminished by 3
mg/kg oral elevation of nociceptive threshold to heat) $(n=6/\text{group}, 3 \text{ tests})$ was not significantly diminished by mg/kg oral (by gavage) dose of suvorexant (actually suvorexant non-significantly increased analges both 5 mg/kg or 10 mg/kg mg/kg oral (by gavage) dose of suvorexant (actually suvorexant non-significantly increased analgesia)
both 5 mg/kg or 10 mg/kg morphine doses (all P=1.00, Tukey post hoc comparisons between morphin
vehicle and suvorexant + mg/kg or 10 mg/kg morphine doses (all P=1.00, Tukey post hoc comparisons between morphine +
vehicle and suvorexant + morphine of either doses). The suvorexant + morphine (5 mg/kg and 10 mg/kg)
analgesic effect vs baseline wehicle and suvorexant + morphine of either doses). The suvorexant + morphine (5 mg/kg and 10 mg/kg) analgesic effect vs baseline was significant (P<0.01 and P<0.001, respectively, Tukey post hoc). The suvorexant dosage (analgesic effect vs baseline was significant (P<0.01 and P<0.001, respectively, Tukey post hoc). The suvorexant dosage (30 mg/kg) that completely preserved opiate analgesia ($\underline{Fig 6}$) is the same as that suvorexant dosage (30 mg/kg) that completely preserved opiate analgesia (Fig 6) is the same as that
surveyed in the same as that the same as that $\frac{1}{2}$ is the same as that the same as that the same as that the same si $s_{\rm s}$ (30 mg/kg) that completely preserved opinate analgesia (Fig.6) is the same as that

in Fig.2, the microglial activation seen in Fig.3 and the morphine anticipation seen in Fig.5.

**P<0.01 and **P<0.001.

We tested the effect of suvorexant on the pain threshold using an IITC PE34

Incremental Thermal Noc in Fig 2, the microglial activation seen in Fig 3 and the morphine anticipation seen in Fig 5. We tested the effect
Incremental Therma
The analgesic effect
seen comparing base
The average analges
(n=6/group, 3 tests)
suvorexant (actually
mg/kg or 10 mg/kg
between morphine a
morphine (5 mg/kg Incremental Thermal Nociceptive Threshold Analgesia Meter (IITC Life Scientive analgesic effect of morphine on the paw raising response to floor heatin
seen comparing baseline (vehicle alone) to 5 mg/kg or 10 mg/kg, morphi The analgesic effect of morphine on the paw raising response to floor heating can be
seen comparing baseline (vehicle alone) to 5 mg/kg or 10 mg/kg, morphine doses.
The average analgesic effect (i.e. elevation of nocicept seen comparing baseline (vehicle alone) to 5 mg/kg or 10 mg/kg, morphine doses.
The average analgesic effect (i.e. elevation of nociceptive threshold to heat)
(n=6/group, 3 tests) was not significantly diminished by the 3 The average analgesic effect (i.e. elevation of nociceptive threshold to heat)
(n=6/group, 3 tests) was not significantly diminished by the 30 mg/kg oral dose of
suvorexant (actually suvorexant non-significantly increased (n=6/group, 3 tests) was not significantly diminished by the 30 mg/kg oral suvorexant (actually suvorexant non-significantly increased analgesia) in bomg/kg or 10 mg/kg morphine doses (all P=1.00, Tukey post hoc, comparis suvorexant (actually suvorexant non-significantly increased analgesia) in both 5
mg/kg or 10 mg/kg morphine doses (all P=1.00, Tukey post hoc, comparison
between morphine and suvorexant + morphine of either doses). The su mg/kg or 10 mg/kg morphine doses (all P=1.00, Tukey post hoc, comparison
between morphine and suvorexant + morphine of either doses). The suvorexant
morphine (5 mg/kg and 10 mg/kg) analgesic effect vs baseline was signifi mg/region morphine and suvorexant + morphine of either doses). The suvorexam
orphine (5 mg/kg and 10 mg/kg) analgesic effect vs baseline was significan
(P<0.01 and P<0.001, respectively, Tukey post-hoc). The suvorexant do morphine (5 mg/kg and 10 mg/kg) analgesic effect vs baseline was significant
(P<0.01 and P<0.001, respectively, Tukey post-hoc).The suvorexant dosage (30
mg/kg) that preserved opiate analgesia is the same as that which
co (P<0.01 and P<0.001, respectively, Tukey post-hoc).The suvorexant dosage (30 mg/kg) that preserved opiate analgesia is the same as that which
completely prevented the chronic morphine associated increase in Hcrt cell nu
a $\rm{G}_{\rm M}$) that preserved opiate analgesia is the same as that which
completely prevented the chronic morphine associated increase in Hcrt cell nun
and decrease in size seen in <u>Fig 2</u>, the microglial activation seen in

Go to:

Discussion

completely prevented the chronic morphine associated increase is
and decrease in size seen in <u>Fig 2</u>, the microglial activation seen in
morphine anticipation seen in <u>Fig 5</u>.
Discussion
As shown in³ and in Fig 1(a), lo and decrease in size seen in Fig 2, the microglial activation seen in Fig 3 and the
morphine anticipation seen in Fig 5.
Discussion
As shown in= and in Fig 1(a), long-term self-administration of heroin in humans or
admin morphine anticipation seen in Fig 5.

Discussion

As shown in³⁴ and in <u>Fig 1(a)</u>, long-term self-administration of heroin in humans

administration of addictive levels of morphine to mice, as well as administration

co Discussion
As shown in²² and in <u>Fig 1(a)</u>, long-te
administration of addictive levels of
cocaine or fentanyl to rats²⁹,²⁰ produc
size of detected Hcrt neurons. We no
antagonist naltrexone prevents these
of Hcrt neu

As shown in a matter in graph only also the non-dimensional or hold in the following or the form in fighting to recaine or fentanyl to ratsa, a produce an increase in the number and decrease in size of detected Hcrt neuro cocaine or fentanyl to rats: $\frac{1}{2}$ are produce an increase in the number and decrease in
size of detected Hcrt neurons. We now show that administration of the opioid
antagonist naltrexone prevents these morphine induc cocaine or fentanyl to rats2, a produce an increase in the number and decrease in
size of detected Hcrt neurons. We now show that administration of the opioid
antagonist naltrexone prevents these morphine induced changes i antagonist naltrexone prevents these morphine induced changes in size and nof Hcrt neurons, confirming that opiate receptor activation is required for this Colchicine, an inhibitor of microtubule polymerization that preve of Hert neurons, confirming that opiate receptor activation is required for this effect.
Colchicine, an inhibitor of microtubule polymerization that prevents transport of
peptides out of the cell soma=, produces an increa Colchicine, an inhibitor of microtubule polymerization that prevents transport of
peptides out of the cell soma_n, produces an increase in the number of detectable
Hcrt neurons, likely due to the accumulation of Hcrt in peptides out of the cell somas, produces an increase in the number of detectable
Hcrt neurons, likely due to the accumulation of Hcrt in the cell soma (Fig 1). The
percentage increase in the number of detected Hcrt neurons peptides out of the cell somas, produces an increase in the number of details Hert neurons, likely due to the accumulation of Hert in the cell soma (<u>Fig 1</u>). The percentage increase in the number of detected Hert neurons percentage increase in the number of detected Hcrt neurons after colchicine is
comparable in magnitude to the increase in the number of Hcrt neurons produce
by chronic administration of heroin in humans or morphine in naïv reprends in magnitude to the increase in the number of Hcrt neurons produce by chronic administration of heroin in humans or morphine in naïve mice or ra suggesting that the opiate induced increase in the number of neurons by chronic administration of heroin in humans or morphine in naïve mice or rats_{a,}
suggesting that the opiate induced increase in the number of neurons staining for
Hcrt results from increased accumulation of Hcrt in neur by chronic administration of heroin in humans of morphine in harve mice or rats..., a, suggesting that the opiate induced increase in the number of neurons staining for Hert results from increased accumulation of Hert in n Hert results from increased accumulation of Hert in neurons that have sub-detecti
levels of Hert under baseline conditions, rather than being caused by neurogenesis
is levels of Hcrt under baseline conditions, rather than being caused by neurogenesis
levels of Hcrt under baseline conditions, rather than being caused by neurogenesis

of "new" Hert neurons.... rrammistration of both morphine and colchicine alone,
increase the number of Hcrt neurons beyond that produced by colchicine alone,
consistent with this conclusions, ∞ .
We have reported that consistent with this conclusion_{^{4, 26},
We have reported that morphine increases Hcrt neuronal projections to the locu
coeruleus. We now show in <u>Fig 4</u>, that 50 mg/kg of morphine for 14 days also
increases projections t} We have reported that morphine in
coeruleus. We now show in Fig 4, t
increases projections to the VTA, a
opiate addiction... We find that He
containing region adjacent to VTA
are not significantly increased by o
projectio coeruleus. We now show in Fig.4, that 50 mg/kg of morphine for 14 days also increases projections to the VTA, a region that has been strongly implicated in opiate addiction......, We find that Hcrt projections to the subs coerations to the VTA, a region fight has been strongly implicated in opiate addictions to the VTA, a region has been strongly implicated in opiate addictions at We find that Hert projections to the substantia nigra, a TH

opiate addiction….. We find that Hert projections to the substantia nigra, a TH containing region adjacent to VTA but not generally implicated in opiate addict
are not significantly increased by opiates, indicating a local opiate addiction-42. We find that Hcrt projections to the substantia nigra, a TH cell
containing region adjacent to VTA but not generally implicated in opiate addiction
are not significantly increased by opiates, indicatin are not significantly increased by opiates, indicating a local regulation of Hcrt
projections.
It has been observed that opiates produce activation of microglial cells throughout
the brain. Microglia have both opioid—and projections.

It has been observed that opiates produce activation of microglial cells through

the brain. Microglia have both opioids and Hcrts receptors. We now report the

suvorexant, a dual Hcrt receptor antagonist, p

provided in thas been of the brain. Mi
suvorexant, of microglia
a key role of
An *in vitro* sl
However ou
increases the opioid agoni
endosomes, the brain. Microglia have both opioids-a and Hcrt-a receptors. We now report that
suvorexant, a dual Hcrt receptor antagonist, prevents morphine induced activation
of microglia in the hypothalamus and in the ventral tegmen the brain. Microglia have both opioid³²² and Hcrie receptors. We now report that
suvorexant, a dual Hcrt receptor antagonist, prevents morphine induced activatio
of microglia in the hypothalamus and in the ventral tegmen of microglia in the hypothalamus and in the ventral tegmental area (Fig 3), showing
a key role of Hcrt in opiate induced microglial proliferation.
An *in vitro* slice study found that opioids decrease the activity of Hcrt a key role of Hcrt in opiate induced microglial proliferation.

An *in vitro* slice study found that opioids decrease the activity of Hcrt neurons».

However our *in vivo* data[®] shows that systemic administration of mor An *in vitro* slice study found that opioids decrease the activit
However our *in vivo* data^{*a*} shows that systemic administration
creases the activity of Hcrt neurons and the brain level of lopioid agonists can be exer However our *in vivo* data¹¹ shows that systemic administration of morphine greatincreases the activity of Hcrt neurons and the brain level of Hcrt+. The effects of opioid agonists can be exerted not only in plasma memb

However our in vivo data 2010 and the brain level of Hert. The effects of
opioid agonists can be exerted not only in plasma membrane receptors and
endosomes, but also in the Golgi apparatus_", suggesting possible intrace increases the activity of Hcrt neurons and the brain rever of the fact, the encoded agonists can be exerted not only in plasma membrane receptors and endosomes, but also in the Golgi apparatus_", suggesting possible intra endosomes, but also in the Golgi apparatus_{¹⁸, suggesting possible intracellula
pathways mediating the opioid induced decrease of Hcrt neuronal size that
reported¹⁸.
Narcoleptic humans, who have an average 90% loss of} endosomes, but also in the Golgi applantates, onggesting possible intracellular pathways mediating the opioid induced decrease of Hcrt neuronal size that we
reported...
Narcoleptic humans, who have an average 90% loss of H preported».

Narcoleptic humans, who have an average 90% loss of Hcrt producing neurons—a,

are resistant to addiction—. Removal of Hcrt neurons in mice significantly reduces

aversion elicited by naloxone precipitated mo Narcoleptic
are resistal
aversion el
role of Hcr
others
receptors r
of detectab
might have
neurons we
Hcrt neuro
dual Hcrt r Narcolepte numans, who have an average 90% loss of Hcrt producing neurons-2-,
are resistant to addiction». Removal of Hcrt neurons in mice significantly reduces
aversion elicited by naloxone precipitated morphine withdraw are resion elicited by naloxone precipitated morphine withdrawal[®]. Because of the role of Hcrt neurons in reward in rodents and pleasure in humans that we₃[±] and others π , π , π have seen, we wanted to rule ou aversion entercate by many different products and pleasure in humans that we₅ μ and others μ , μ , μ have seen, we wanted to rule out the possibility that blocking Hert receptors might also affect the opiate el role of Hcrt neurons in reward in rodents and pleasure in humans that we₅.4 and
others_{^{19, 19}, ² a have seen, we wanted to rule out the possibility that blocking Hcrt
receptors might also affect the opiate elicited s} others_{2, 2,} 2 have seen, we wanted to rule out the possibility that blocking rict receptors might also affect the opiate elicited shrinkage and increase in the nu of detectable Hcrt neurons that we discovered. We thought of detectable Hcrt neurons that we discovered..., We thought that this blockade might have no effect, since we assumed that the morphine induced changes in Hcrt neurons were solely the result of direct opioid action on mu of detectable Hert heat ons that we discovered. We thought that this blockade
might have no effect, since we assumed that the morphine induced changes in I
neurons were solely the result of direct opioid action on mu opioi meurons were solely the result of direct opioid action on mu opioid receptors on
Hcrt neurons (Fig 1). However, to our surprise, we found that administration of the
dual Hcrt receptor antagonist completely prevented the in Hert neurons (Fig 1). However, to our surprise, we found that administration of t
dual Hert receptor antagonist completely prevented the increase in number and
decrease in size of Hert neurons produced by opiate administr Hacker Hart receptor antagonist completely prevented the increase in number and the decrease in size of Hcrt neurons produced by opiate administration (Fig 2). This finding led us to further test the effect of suvorexant o decrease in size of Hcrt neurons produced by opiate administration (Fig 2). This
finding led us to further test the effect of suvorexant on morphine anticipation and
on analgesia. decrease in the crease in the crease in produced by opinate administration (Fig 2). This
finding led us to further test the effect of suvorexant on morphine anticipation are
on analgesia. finding led us to further the effect of substitution and product of subsets and $\frac{1}{2}$ and $\$ on analgesia.

We utilized a where running model of addictive anticipated these injections by vigorous running starting at ZT2, at morphine doses as low as 5 mg/kg (Fig 5). However when morphine injections were preceded by suvorexant ad injections by vigorous running starting at ZT2, at morphine doses as low as 5 (Fig 5). However when morphine injections were preceded by suvorexant administration no anticipatory wheel running occurred. Note that the anti (Fig 5). However when morphine injections were preceded by suvorexant administration no anticipatory wheel running occurred. Note that the anticipation was occurring 22 hours after the prior suvorexant injection 21 hours administration no anticipatory wheel running occurred. Note that the anticus occurring 22 hours after the prior suvorexant injection 21 hours after morphine injection and 3 hours before the next morphine injection, at a t was occurring 22 hours after the prior suvorexant injection 21 hours after the prio
morphine injection and 3 hours before the next morphine injection, at a time when
minimal amounts of morphine or suvorexant would still be morphine injection and 3 hours before the next morphine injection, at a time when
minimal amounts of morphine or suvorexant would still be present in the mice. It
seems likely that it was the presence of suvorexant when th minimal amounts of morphine or suvorexant would still be present in the mice. It
seems likely that it was the presence of suvorexant when the morphine was injectee
that prevented the condtioned anticipatory running 22 hour seems likely that it was the presence of suvorexant when the morphine was inject
that prevented the condtioned anticipatory running 22 hours later. The suvorexan
effect on morphine anticipation may be a result of the block that prevented the condtioned anticipatory running 22 hours later. The suvorexant effect on morphine anticipation may be a result of the blockade of self-excitation of the Hcrt neuronal populations, \approx This blockade of H

effect on morphine anticipation may be a result of the blockade of self-excitation of
the Hcrt neuronal population_{^{8, a}} This blockade of Hcrt receptors appears to provide
the same protection against substance use disord the Hert neuronal population_{^{3, a}} This blockade of Hert receptors appears to provide
the same protection against substance use disorder that is present in the absence of
Hert neurons in human and animal narcoleptics and the Hert neuronal populations, 2 This blockade of Hcrt receptors appears to provide
the same protection against substance use disorder that is present in the absence of
Hcrt neurons in human and animal narcoleptics and aft Hert neurons in human and animal narcoleptics and after naloxone administration (Fig 1).
At the suvorexant dose used, even though given in the light period (the normal sleep
period) no sleep occurred in either the mice giv (Fig 1).

At the suvorexant dose used, even though given in the light period (the normal slee

period) no sleep occurred in either the mice given morphine or the mice given

suvorexant + morphine, as evidenced by wheel ru At the s
period)
suvorex
is due to
even the
recepto
recepto
did not
Hcrt cel
itself is
Sleepine period) no sleep occurred in either the mice given morphine or the mice given
suvorexant + morphine, as evidenced by wheel running and video observation. This
is due to the well-known arousing quality of opioids in mice an suvorexant + morphine, as evidenced by wheel running and video observation.
is due to the well-known arousing quality of opioids in mice and rats=. Therefore
even though suvorexant is a "sleeping pill" at certain doses in is due to the well-known arousing quality of opioids in mice and rats». Therefore,
even though suvorexant is a "sleeping pill" at certain doses in humans (the dual Hcrt
receptor antagonist suvorexant is marketed as Belsom is due to the well-known arousing quality of operation the dual Hirecteptor antagonist suvorexant is a "sleeping pill" at certain doses in humans (the dual Hirecteptor antagonist suvorexant is marketed as Belsomra, with n receptor antagonist suvorexant is marketed as Belsomra, with newer dual Hcrt
receptor antagonists daridorexant in Quviviq and lemborexant in Dayvigo), sleep
did not mediate the observed effect of this drug on morphine indu

receptor antagonists daridorexant in Quviviq and lemborexant in Dayvigo), slee
did not mediate the observed effect of this drug on morphine induced changes i
Hcrt cell number and size shown in <u>Figs 1</u>&2. It is useful to r did not mediate the observed effect of this drug on morphine induced changes in
Hert cell number and size shown in <u>Figs 1</u>&2. It is useful to recall that morphine
itself is soporific in humans, which is why morphine was n Hert cell number and size shown in <u>Figs 1</u>&2. It is useful to recall that morphine
itself is soporific in humans, which is why morphine was named after Morpheus.
Sleepiness does not prevent the opiate addictive process₂ itself is soporific in humans, which is why morphine was named after Morpheus
Sleepiness does not prevent the opiate addictive process».
Suvorexant prevents the changes in Hcrt neuron number and size, produced by
chronic Sleepiness does not prevent the opiate addictive process

Sleepiness does not prevent the opiate addictive process

Suvorexant prevents the changes in Hcrt neuron number and size, produced by

chronic administration of op Suvorexant prevents the changes in Hcrt neuron number a
chronic administration of opiates (Fig 2), the microglial act
the anticipatory motor activation in mice expecting opioid
However, we find that opiate analgesia is not chronic administration of opiates (<u>Fig 2</u>), the microglial activation seen in <u>Fig 3</u>, the anticipatory motor activation in mice expecting opioid administration (Fig 5 However, we find that opiate analgesia is not at all the anticipatory motor activation in mice expecting opioid administration (Fig 5).
However, we find that opiate analgesia is not at all diminished by suvorexant (Fig 6)
These findings suggest that administration of Hcrt r However, we find that opiate analgesia is not at all diminished by suvorexant $\left(\frac{Fig}{Fig}\right)$.
These findings suggest that administration of Hcrt receptor antagonists combined with opioids for pain relief in humans may gr These findings suggest that administration of Hert receptor antagonists combined
with opioids for pain relief in humans may greatly reduce addiction risk and
consequent morbidity while providing maximal analgesia.

<u>Go to:</u> with opioids for pain relief in humans may greatly reduce addiction risk and
consequent morbidity while providing maximal analgesia.
<u>Go</u>
Methods where the providing maximal analgesia.
We discussed in the human relief in the human reduce and the human reduce and the methods of the methods of the state and the state and the methods of the state and the state and the consequent more more in the providing maximal and the providing maximal and solven in the providing maximal and $\frac{1}{2}$ an

Go to: \ddot{a}

Methods

Animal Usage

of the University of California at Los Angeles and of the Veterans Administration
Greater Los Angeles Health Care System. Experiments were performed in C57BL/6
mice. A total of 151 male mice were used in the current study Greater Los Angeles Health Care System. Experiments were performed in C57BL
mice. A total of 151 male mice were used in the current study. All experimental
procedures were started when animals reached 3 months of age. Anim

Drugs

mice. A total of 151 male mice were used in the current study. All experimental
procedures were started when animals reached 3 months of age. Animals were kep
in a room maintained at 22±1°C on a 12 h light (135 lux) dark (procedures were started when animals reached 3 months of age. Animals were
in a room maintained at 22±1°C on a 12 h light (135 lux) dark (0.03 lux) cycle (l
on at 7 AM and off at 7 PM) or on a "skeleton" light schedule as procedure in a room maintained at 22±1°C on a 12 h light (135 lux) dark (0.03 lux) cycle (lights
on at 7 AM and off at 7 PM) or on a "skeleton" light schedule as described below.
Drugs
Morphine sulfate (Hospira Inc., Lak in a room at 7 AM and off at 7 PM) or on a "skeleton" light schedule as described below.

Drugs
 Morphine sulfate (Hospira Inc., Lake Forest, IL, USA) and **Naltrexone**
 hydrochloride, (Sigma- Aldrich, N3136, Lot # SLBF Drugs
 Morphine sulfate (Hospira Inc., Lake Forest, IL, USA) and **Naltrexone**
 hydrochloride, (Sigma- Aldrich, N3136, Lot # SLBF858548, Saint Louis, MO, USA

50 mg/kg were used. Morphine and naltrexone were dissolved i Morphine sunate (Hospira Inc., Lake Forest, LE, ObA) and Natit Coole
hydrochloride, (Sigma- Aldrich, N3136, Lot # SLBF858548, Saint Louis
50 mg/kg were used. Morphine and naltrexone were dissolved in steril
immediately bef

Tissue processing

Hydrochloride, (Sigma- Aldrich, N3136, Lot # SLBF85853-0, Saint Louis, MO, OSA)
50 mg/kg were used. Morphine and naltrexone were dissolved in sterile saline
immediately before subcutaneous administration. **Suvorexant** (Bel immediately before subcutaneous administration. **Suvorexant** (Belsomra, Mer
USA) was suspended in 0.5% methylcellulose in water and orally administered
gavage.
Tissue processing
Animals were anesthetized intraperitoneally immediately before subcutaneous administration. Suvorexant (Bersonica, Merck NJ,
USA) was suspended in 0.5% methylcellulose in water and orally administered by
gavage.
Tissue processing
Animals were anesthetized intraperit Example 2.1 and the sum of the solution (150 mg/kg) and then perfused transcardially with PBS (0.1M, pH 7.4), followed by 4% formaldehyde in PBS. Brains were removed Tissue
Tissue
Animals
solution
followed
hogether
three-se
immedia
stored a
together solution (150 mg/kg) and then perfused transcardially with PBS (0.1M, pH 7.4),
followed by 4% formaldehyde in PBS. Brains were removed and post-fixed for 7
hours in 4% formaldehyde in PBS, followed by 20% sucrose in PBS fo followed by 4% formaldehyde in PBS. Brains were removed and post-fixed for 7.
hours in 4% formaldehyde in PBS. Brains were removed and post-fixed for 7.
hours in 4% formaldehyde in PBS, followed by 20% sucrose in PBS for follower in 4% formaldehyde in PBS, followed by 20% sucrose in PBS for 24 hours and 30% for 48 hours. Brains were frozen and cut into 40 μm coronal sections using a sliding microtome (American Optical, USA). The sections 30% for 48 hours. Brains were frozen and cut into 40 µm coronal sections using a
sliding microtome (American Optical, USA). The sections were sorted into one in
sliding microtome (American Optical, USA). The sections were sliding microtome (American Optical, USA). The sections were sorted into one in
three-section compartments. Immunohistochemical procedures were performed
immediately. The remaining tissue was transferred to a cryoprotecta three-section compartments. Immunohistochemical procedures were performed
immediately. The remaining tissue was transferred to a cryoprotectant solution a
stored at –20°C. Mice that we compared were always sacrificed and

Immunostaining for brightfield microscopy

immediately. The remaining tissue was transferred to a cryoprotectant solution a
stored at –20°C. Mice that we compared were always sacrificed and processed
together. Perfusion, histology and microscopic analysis were per stored at –20°C. Mice that we compared were always sacrificed and processed
together. Perfusion, histology and microscopic analysis were performed by
investigators blind to the procedures performed prior to sacrifice.
Immu together. Perfusion, histology and microscopic analysis were performed by
investigators blind to the procedures performed prior to sacrifice.
Immunostaining for brightfield microscopy
All immunohistochemical procedures we investigators blind to the procedures performed prior to sacrifice.
Immunostaining for brightfield microscopy
All immunohistochemical procedures were performed by sequential incuba
free-floating sections. For detection of Immunostaining for brightfield microscopy
All immunohistochemical procedures were performed by sequentif
free-floating sections. For detection of Hcrt and MCH, sections wer
for 30 min in 0.5% H_2O_2 in PBS to bloc Free-floating sections. For detection of Hcrt and MCH, sections were first incubated
for 30 min in 0.5% H_2O_2 in PBS to block endogenous peroxidase activity. After
thorough washing with PBS, the sections were placed fo for 30 min in 0.5% H_2O_2 in PBS to block endogenous peroxidase activity. After thorough washing with PBS, the sections were placed for 2 hours in 1.5% normal goat serum (NGS) in PBS containing 0.25% Triton X (PBST) and thorough washing with PBS, the sections were placed for 2 hours in 1.5% norr
goat serum (NGS) in PBS containing 0.25% Triton X (PBST) and incubated for
 goat serum (NGS) in PBS containing 0.25% Triton X (PBST) and incubated for 72 $\frac{1}{2}$ goat serum (NGS) in PBS containing 0.25% Triton X (PBST) and incubated for 72

(1:10000, H-003-30, Lot # 01108, Phoenix Pharmaceuticals Inc.) or rabbit anti-
(1:20000, H-070-47, Lot # 01629-5, Phoenix Pharmaceuticals Inc.), followed by
corresponding biotinylated secondary antibody (1:400, Vector Lab (1:20000, H-070-47, Lot # 01629-5, Phoenix Pharmaceuticals Inc.), followed by the corresponding biotinylated secondary antibody (1:400, Vector Laboratories)in PBST for 2 h, and avidin- biotin-peroxidase complex (1:300, AB corresponding biotinylated secondary antibody (1:400, Vector Laboratories)in
PBST for 2 h, and avidin-biotin-peroxidase complex (1:300, ABC Elite Kit, Vector
Laboratories) in PBS for 2 h. The tissue-bound peroxidase was th PBST for 2 h, and avidin- biotin-peroxidase complex (1:300, ABC Elite Kit, Vectorresponding to the diaminobenzidine tetrahydrochloride (DAB) method, which consisted of tis immersion in 0.02% DAB and 0.03% hydrogen peroxida Laboratories) in PBS for 2 h. The tissue-bound peroxidase was then developed us
the diaminobenzidine tetrahydrochloride (DAB) method, which consisted of tissu
immersion in 0.02% DAB and 0.03% hydrogen peroxide in 10 ml PB the diaminobenzidine tetrahydrochloride (DAB) method, which consisted of tissue
immersion in 0.02% DAB and 0.03% hydrogen peroxide in 10 ml PBS. Microglia
were identified using the canonical marker, ionized calcium bindin immersion in 0.02% DAB and 0.03% hydrogen peroxide in 10 ml PBS. Microglia
were identified using the canonical marker, ionized calcium binding adaptor
molecule-1 (Iba-1)=. Prior to Iba-1 immunostaining, an antigen retrieva were identified using the canonical marker, ionized calcium binding adaptor
molecule-1 (Iba-1)_a. Prior to Iba-1 immunostaining, an antigen retrieval procedu
was performed by incubating the sections in 10mM sodium citrate molecule-1 (Iba-1)². Prior to Iba-1 immunostaining, an antigen retrieval procuss performed by incubating the sections in 10mM sodium citrate (pH 8.5) a
for 30 min. The sections were then cooled to room temperature in so

molecule -1 (in 1)-1 (in the sections in 10mM sodium citrate (pH 8.5) at 80°C
for 30 min. The sections were then cooled to room temperature in sodium citrate,
for 30 min. The sections were then cooled to room temperature for 30 min. The sections were then cooled to room temperature in sodium citrate, washed with PBS and following the same staining procedures used to detect Hcrt-1 using primary antibody goat anti-Iba-1 (ab5076, Abcam, Lot#G washed with PBS and following the same staining procedures used to detect Hcrtusing primary antibody goat anti-Iba-1 (ab5076, Abcam, Lot#GR3403958, 1:1000
and normal rabbit serum.
We previously standardized our DAB method using primary antibody goat anti-Iba-1 (ab5076, Abcam, Lot#GR3403958, 1:10000)
and normal rabbit serum.
We previously standardized our DAB method and established an 8-minute optimal
developing time and used this precise du and normal rabbit serum.
We previously standardized our DAB method and established an 8-minute optimal
developing time and used this precise duration in all of our studies. All developing
solutions were prepared in one con We previously standardize
developing time and used
solutions were prepared in
respective developing wel
lights off and the wells cor
protect them from light ex
solutions were used only of
Brightfield microscopy
The number, developing time and used this precise duration in all of our studies. All developing
solutions were prepared in one container, homogenized and aliquoted to the
respective developing wells. Developing procedures were perfor solutions were prepared in one container, homogenized and aliquoted to the
respective developing wells. Developing procedures were performed with room
lights off and the wells containing tissue were wrapped with aluminum f

Brightfield microscopy

respective developing wells. Developing procedures were performed with ro
lights off and the wells containing tissue were wrapped with aluminum foil to
protect them from light exposure. Wells were agitated at 55 rpm. Devel lights off and the wells containing tissue were wrapped with aluminum foil to
protect them from light exposure. Wells were agitated at 55 rpm. Developing
solutions were used only once.
Brightfield microscopy
The number, d protect them from light exposure. Wells were agitated at 55 rpm. Developing
solutions were used only once.
Brightfield microscopy
The number, distribution and size of Hcrt+ and Ibal-1+ cells and the number a
distribution o provided microscopy

Prightfield microscopy

Prightfield microscopy

The number, distribution and size of Hcrt+ and Ibal-1+ cells and the number a

distribution of MCH were assessed using a Nikon Eclipse 80i microscope wi Brightfield microscopy
The number, distribution and s
distribution of MCH were asses
axis motorized stage, video can
software (MicroBrightField Cor
either 40x or 60x objective and
Nucleator probe. Iba-1+ quantif
the Hcrt n distribution of MCH were assessed using a Nikon Eclipse 80i microscope with thr
axis motorized stage, video camera, Neurolucida interface and Stereoinvestigator
software (MicroBrightField Corp.). Cell counting was performe axis motorized stage, video camera, Neurolucida interface and Stereoinvestigator software (MicroBrightField Corp.). Cell counting was performed bilaterally using either 40x or 60x objective and cell size was determined usi software (MicroBrightField Corp.). Cell counting was performed bilaterally using
either 40x or 60x objective and cell size was determined using the Neurolucida
Nucleator probe. Iba-1+ quantification was performed bilatera either 40x or 60x objective and cell size was determined using the Neurolucida
Nucleator probe. Iba-1+ quantification was performed bilaterally in the middle of
the Hcrt neuronal field by placing a square (250 μ m x 250 Nucleator probe. Iba-1+ quantification was performed bilaterally in the middle
the Hcrt neuronal field by placing a square (250 μ m x 250 μ m) dorsal to the form
with the lower corners of the square equidistant from t the Hcrt neuronal field by placing a square (250 µm x 250 µm) dorsal to the fornix
with the lower corners of the square equidistant from the center of the fornix.
Quantification of lba-1+ cells was performed bilaterally in with the lower corners of the square equidistant from the center of the fornix.
Quantification of Iba-1+ cells was performed bilaterally in the same manner in the
VTA, by placing a square (250 μm x 250 μm) medial to the m Quantification of Iba-1+ cells was performed bilaterally in the same manner in
VTA, by placing a square (250 µm x 250 µm) medial to the medial lemniscus. A
counting and cell measurements were performed by a trained histolo VTA, by placing a square (250 μ m x 250 μ m) medial to the medial lemniscus. All counting and cell measurements were performed by a trained histologist, always blind to the experimental condition. In every case, the s Value of the specifical measurements were performed by a trained histologist, always
blind to the experimental condition. In every case, the same individual counted b
the experimental and control tissue. Only neurons with counted to the experimental condition. In every case, the same individual counted bothe experimental and control tissue. Only neurons with an identifiable nucleus we counted. In addition to quantitative assessments, we loo the experimental and control tissue. Only neurons with an identifiable nucleus were
counted. In addition to quantitative assessments, we look for nuclear fragmentation, the experimental and control time in the expendence of the counted. In addition to quantitative assessments, we look for nuclear fragmentation,
counted. In addition to quantitative assessments, we look for nuclear fragment counted. In addition to quantitative assessments, we look for nuclear fragmentation, we look for nuclear fragm
In addition, we look for nuclear fragmentation, we look for nuclear fragmentation, we look for nuclear fragmen

Immunostaining for confocal microscopy

chromatolysis, inclusions, variosities and other abnormalities, 2,2,2, but we did not
see these phenomena in the current study.

Immunostaining for confocal microscopy

For identification of dopamine (DA) containing neuron Immunostaining for confocal microscopy
For identification of dopamine (DA) contain
nigra (SN) regions we used the immunohis
sections were first incubated in PBST conta
donkey serum (NDS), followed by co-incub
anti-Hcrt-1 (nigra (SN) regions we used the immunohistochemical detection of TH enzyme. The
sections were first incubated in PBST containing 1.5% of both NGS and normal
donkey serum (NDS), followed by co-incubation with primary antibod nections were first incubated in PBST containing 1.5% of both NGS and normal
donkey serum (NDS), followed by co-incubation with primary antibodies rabbit
anti-Hcrt-1 (H-003-36, Phoenix Pharmaceuticals, USA, 1:2000, Lot # 0 donkey serum (NDS), followed by co-incubation with primary antibodies rabbit
anti-Hcrt-1 (H-003-36, Phoenix Pharmaceuticals, USA, 1:2000, Lot # 01108) an
sheep anti-TH (ab113, Abcam, USA, 1:1000, Lot # GR 3277795-15) overn anti-Hcrt-1 (H-003-36, Phoenix Pharmaceuticals, USA, 1:2000, Lot # 01108) and
sheep anti-TH (ab113, Abcam, USA, 1:1000, Lot # GR 3277795-15) overnight at
room temperature in PBST. Next, we washed the sections and incubate sheep anti-TH (ab113, Abcam, USA, 1:1000, Lot # GR 3277795-15) overnight at
room temperature in PBST. Next, we washed the sections and incubated them in
PBST containing the corresponding secondary antibody tagged with fluo room temperature in PBST. Next, we washed the sections and incubated them in
PBST containing the corresponding secondary antibody tagged with fluorophore
that match our microscope filters (1:300, Alexafluor 488 goat anti-r PBST containing the corresponding secondary antibody tagged with fluorophore that match our microscope filters (1:300, Alexafluor 488 goat anti-rabbit, A11001
Lot # 2557379, Alexafluor 555 donkey anti-sheep, A 21436, Lot # that match our microscope filters (1:300, Alexafluor 488 goat anti-rabbit, A11008,
Lot # 2557379, Alexafluor 555 donkey anti-sheep, A 21436, Lot # 2420712
ThermoFisher Scientific, US), 1% NGS and 1% NDS, with lights off a Lot # 2557379, Alexafluor 555 donkey anti-sheep, A 21436, Lot # 2420712
ThermoFisher Scientific, US), 1% NGS and 1% NDS, with lights off and samples
wrapped in aluminum foil. Tissue was mounted and cover slipped using Vect ThermoFisher Scientific, US), 1% NGS and 1% NDS, with lights off and samp
wrapped in aluminum foil. Tissue was mounted and cover slipped using Vec
Shield anti fade mounting media (H 1000, Vector Laboratories, Burlingame,
 wrapped in aluminum foil. Tissue was mounted and cover slipped using Vector
Shield anti fade mounting media (H 1000, Vector Laboratories, Burlingame, CA,
Lot # 2E00806). All tissue sections from experimental and control a Shield anti fade mounting media (H 1000, Vector Laboratories, Burlingame, CA,
Lot # 2E00806). All tissue sections from experimental and control animals were
stained at the same time and with the same antibody lot. To quant Lot # 2E00806). All tissue sections from experimental and control animals were
stained at the same time and with the same antibody lot. To quantify the number of
TH+ neurons, the same mounting media containing 42, 6-diamid stained at the same time and with the same antibody lot. To quantify the number
TH+ neurons, the same mounting media containing 42, 6-diamidino-2-phenylind
(DAPI) was used (H 1200, Vector Laboratories, Burlingame, CA, USA,

Confocal microscopy

TH+ neurons, the same mounting media containing 42, 6-diamidino-2-phenylindole
(DAPI) was used (H 1200, Vector Laboratories, Burlingame, CA, USA, Lot # 2E0815)
All tissue sections from experimental and control animals wer (DAPI) was used (H 1200, Vector Laboratories, Burlingame, CA, USA, Lot # 2E0815).
All tissue sections from experimental and control animals were stained at the same
time and with the same antibody lot.
Confocal microscopy (A) Hissue sections from experimental and control animals were stained at the same
time and with the same antibody lot.

Confocal microscopy

The number and distribution of Hcrt fibers and TH cell bodies was assessed usin The number and distribution of Hcrt fibers and TH cell bodies was assessed using a
The number and distribution of Hcrt fibers and TH cell bodies was assessed using a
Zeiss LSM 900 (Imager Z2 AX10, Jena, Germany) confocal Confocal microscopy

The number and distribution of Hcrt

Zeiss LSM 900 (Imager Z2 AX10, Jena

the appropriate lasers. Every section

imaged at 1 µm optical planes; 28 ± 1

respectively. Quantification was perf

throughou Zeiss LSM 900 (Imager Z2 AX10, Jena, Germany) confocal microscope equipped with
the appropriate lasers. Every section that contained the VTA and adjacent SN was
imaged at 1 µm optical planes; 28 ± 1.4 optical planes were the appropriate lasers. Every section that contained the VTA and adjacent SN was imaged at 1 µm optical planes; 28 ± 1.4 optical planes were obtained per section respectively. Quantification was performed bilaterally on imaged at 1 μ m optical planes; 28 ± 1.4 optical planes were obtained per section
respectively. Quantification was performed bilaterally on every third section
throughout the region of interest. Immunofluorescence inten respectively. Quantification was performed bilaterally on every third section
throughout the region of interest. Immunofluorescence intensities and area
measurements were obtained using the Zeiss proprietary software ZEN®. throughout the region of interest. Immunofluorescence intensities and area
measurements were obtained using the Zeiss proprietary software ZEN®. The
was defined by the size of the region containing TH neuronal bodies in ea throughoutements were obtained using the Zeiss proprietary software ZEN®. Th
was defined by the size of the region containing TH neuronal bodies in each
structure. Total immunofluorescence for TH and Hcrt was divided by th measurements were obtained using the Zeiss proprietary software ZEN®. The area
was defined by the size of the region containing TH neuronal bodies in each
structure. Total immunofluorescence for TH and Hcrt was divided by structure. Total immunofluorescence for TH and Hcrt was divided by the
corresponding area, and bilateral areas in the same section were averaged. (
number and fiber distribution were determined using the Adobe Illustrator
 corresponding area, and bilateral areas in the same section were average
number and fiber distribution were determined using the Adobe Illustrat
program. Every stack of images was loaded in this program such that each
plan number and fiber distribution were determined using the Adobe Illustrator
program. Every stack of images was loaded in this program such that each optic
plane was placed in a distinct layer and individual TH containing neu number and fiberal fiberal fiber and fiberal conditions were determined using the Adobe Incorporation were plane was placed in a distinct layer and individual TH containing neurons were determined using the Adobe III and t plane was placed in a distinct layer and individual TH containing neurons were
plane was placed in a distinct layer and individual TH containing neurons were plane was placed in a distinct layer and individual TH containing neurons were

counting of cells, which is critical in the analysis of structures with a high denomely and meuronal bodies like the VTA and SN.
For the analysis of Hcrt fiber length and distribution, the middle optical plane
chosen for neuronal bodies like the VTA and SN.

For the analysis of Hcrt fiber length and distribution, the middle optical plane was

chosen for matching sections that contained the VTA. Individual fibers were drawn

and measured a

Colchicine procedure

For the analysis of Hcrt fiber length and
chosen for matching sections that con
and measured and the total fiber leng
to calculate the immunofluorescence
Colchicine procedure
Two groups of animals (n=4 per grou
(ICV) injec For the analysis of matching sections that contained the VTA. Individual fibers were drawn
and measured and the total fiber length was then calculated based on the area use
to calculate the immunofluorescence intensities. and measured and the total fiber length was then calculated based on the area used
to calculate the immunofluorescence intensities.
Colchicine procedure
Two groups of animals (n=4 per group) were subjected to intracerebro to calculate the immunofluorescence intensities.

Colchicine procedure

Two groups of animals (n=4 per group) were subjected to intracerebroventricular

(ICV) injection of either saline solution or saline solution contain Colchicine procedure
Two groups of animals (n=4 per group) were sub
(ICV) injection of either saline solution or saline :
colchicine, a microtubule disruptor that prevents
levels of neuropeptides in cell somas… Another ty
 (ICV) injection of either saline solution or saline solution containing 20 μ g/ μ of colchicine, a microtubule disruptor that prevents peptide transport and increases levels of neuropeptides in cell somas... Another t colchicine, a microtubule disruptor that prevents peptide transport and increase
levels of neuropeptides in cell somas... Another two groups of animals (n=4 per
group) received 14 d of either saline or morphine (50mg/kg) levels of neuropeptides in cell somas... Another two groups of animals (n=4 per
group) received 14 d of either saline or morphine (50mg/kg) before ICV injections
of colchicine. An additional 4 naïve animals served as the group) received 14 d of either saline or morphine (50mg/kg) before ICV injectiof of colchicine. An additional 4 naïve animals served as the control. Anesthesia wainduced with a mixture of ketamine/xylazine (100 mg/kg/15 m group) and intertains a matter of either and the saling the saling of excellent and the saling of colchicine. An additional 4 naïve animals served as the control. Anesthesia was induced with a maintained with a gas mixtur induced with a mixture of ketamine/xylazine (100 mg/kg/15 mg/kg, i.p.) and the
maintained with a gas mixture of isoflurane in oxygen (1-3%) after the animals
were placed in the stereotaxic device. Body temperature was mai maintained with a gas mixture of isoflurane in oxygen $(1-3\%)$ after the animals
were placed in the stereotaxic device. Body temperature was maintained with a
water-circulating heating pad (Gaymar Industries, Orchard Park were placed in the stereotaxic device. Body temperature was maintained with a water-circulating heating pad (Gaymar Industries, Orchard Park, NY, USA). The was positioned in a stereotaxic frame and the skull was exposed. water-circulating heating pad (Gaymar Industries, Orchard Park, NY, USA). The lwas positioned in a stereotaxic frame and the skull was exposed. A hole was dril at coordinates corresponding to the lateral ventricle (AP: -0 was positioned in a stereotaxic frame and the skull was exposed. A hole was drilled
at coordinates corresponding to the lateral ventricle (AP: –0.5 mm, L: –1 mm,
relative to bregma). A Hamilton microsyringe was lowered unt at coordinates corresponding to the lateral ventricle (AP: -0.5 mm, L: -1 mm, relative to bregma). A Hamilton microsyringe was lowered until the ventricle was reached (H-2.8 mm, relative to the skull surface). Infusio relative to bregma). A Hamilton microsyringe was lowered until the ventricle reached (H-2.8 mm, relative to the skull surface). Infusion was made in increment of 0.2 μl every 10 minutes for 40 minutes to obtain a final v reached (H-2.8 mm, relative to the skull surface). Infusion was made in increments
of 0.2 μ l every 10 minutes for 40 minutes to obtain a final volume of 1 μ l. The need
was held in place for another 10 minutes before of 0.2 μ every 10 minutes for 40 minutes to obtain a final volume of 1 μ l. The needlems held in place for another 10 minutes before being slowly withdrawn.
Ventricular localization was confirmed by observing free fl was held in place for another 10 minutes before being slowly withdrawn.
Ventricular localization was confirmed by observing free flowing cerebrospinal fluid
after the withdrawal of the needle. A small piece of sterile bon Ventricular localization was confirmed by observing free flowing cerebros
after the withdrawal of the needle. A small piece of sterile bone wax was p
the hole and the skin sutured. All subjects recovered from the anesthes

Morphine anticipation

after the withdrawal of the needle. A small piece of sterile bone wax was placed over
the hole and the skin sutured. All subjects recovered from the anesthesia within 30
minutes after the end of the procedure. Animals were the hole and the skin sutured. All subjects recovered from the anesthesia within 30
minutes after the end of the procedure. Animals were carefully monitored and
sacrificed 52 h later between ZT 13 and ZT 15 for immunohisto minutes after the end of the procedure. Animals were carefully monitored and sacrificed 52 h later between ZT 13 and ZT 15 for immunohistochemical procedure.
Morphine anticipation
To measure conditioned addictive anticipa sacrificed 52 h later between ZT 13 and ZT 15 for immunohistochemical procedure.
Morphine anticipation
To measure conditioned addictive anticipation, Juarez-Portilla et al.* developed
wheel running test in mice. This techn Morphine anticipation
To measure conditioned addictive anticipation, Juarez-Portilla et al.^{*} developed a
wheel running test in mice. This technique has been used to quantify drug
anticipation and appetitive changes..., W To measure conditioned address anticipation, Juarez-Portina et al. actriciped a
wheel running test in mice. This technique has been used to quantify drug
anticipation and appetitive changes a. We adapted this method for me anticipation and appetitive changes₂. We adapted this method for measuri
morphine anticipation. The running wheels were low-profile running whe
mice, placed inside the testing cages, L 48.3 cm, W 26.7 cm and H 40.6 cm a anticipation and appetitive changes. We adapted this include for measuring
morphine anticipation. The running wheels were low-profile running wheels
mice, placed inside the testing cages, L 48.3 cm, W 26.7 cm and H 40.6 cm mice, placed inside the testing cages, L 48.3 cm, W 26.7 cm and H 40.6 cm and $\frac{1}{2}$ $\frac{1}{\sqrt{1-\frac{1$

to a "skeleton light cycle" (lights on at 6:00 AM and off at 6:00 PM, 60 min total light
per 24 h period, light was on for 30 min at the beginning of the "light" period and 30
min at the end of the 12 h "light" cycle) for per 24 h period, light was on for 30 min at the beginning of the "light" period and 30 min at the end of the 12 h "light" cycle) for 4 weeks. This lighting schedule disinhibits running wheel behavior during a period in whi min at the end of the 12 h "light" cycle) for 4 weeks. This lighting schedule
disinhibits running wheel behavior during a period in which light would otherwise
inhibit wheel running, then running wheels were introduced fo disinhibits running wheel behavior during a period in which light would of
inhibit wheel running, then running wheels were introduced for an additio
days before the start of drug administration. Running wheel activity was inhibit wheel running, then running wheels were introduced for an additional 14
days before the start of drug administration. Running wheel activity was collected
and analyzed using Med Associates software (SOF-861). Conti days before the start of drug administration. Running wheel activity was collected and analyzed using Med Associates software (SOF-861). Continuous video monitoring and the wheel running record showed that no sleep occurre and analyzed using Med Associates software (SOF-861). Continuous video
monitoring and the wheel running record showed that no sleep occurred for more
than 4 h after morphine administration in any mouse.
A total of 36 male

Analgesia measurement

monitoring and the wheel running record showed that no sleep occurred for
than 4 h after morphine administration in any mouse.
A total of 36 male mice received 14 days of orally administered suvorexant
mg/kg) or vehicle (0 than 4 h after morphine administration in any mouse.

A total of 36 male mice received 14 days of orally administered suvorexant (30

mg/kg) or vehicle (0.5% methylcellulose) at 10:00 AM (ZT4) followed by morphine

(5 or 1 A total of 36 male mice received 14 days of orally adm
mg/kg) or vehicle (0.5% methylcellulose) at 10:00 AM
(5 or 10 mg/kg, subcutaneous) or saline (0.05 ml, subd
Analgesia measurement
We measured the effect of morphine wi mg/kg) or vehicle (0.5% methylcellulose) at 10:00 AM (ZT4) followed by morpl(5 or 10 mg/kg, subcutaneous) or saline (0.05 ml, subcutaneous) at 11:00 AM (2)
Analgesia measurement
We measurement
We measurement
We measurement (5 or 10 mg/kg, subcutaneous) or saline (0.05 ml, subcutaneous) at 11:00 AM (ZT5)
Analgesia measurement
We measured the effect of morphine with and without suvorexant on the pain
threshold using an IITC PE34 Incremental T (5 or 10 mg/kg, subcurring) or saling (1.05 mg/kg, subcurring) at 22.05).

We measured the effect of morphine with and without suvorexant on the pain

threshold using an IITC PE34 Incremental Thermal Nociceptive Threshold threshold using an IITC PE34 Incremental Thermal Nociceptive Threshold Ana
Meter (IITC Life Science Inc), which raises the temperature of its aluminum suat 6°C/min in each trial. When the mouse licked or shook a hindlimb Meter (IITC Life Science Inc), which raises the temperature of its aluminum surface
at 6°C/min in each trial. When the mouse licked or shook a hindlimb or jumped, the
experimenter immediately removed it from the apparatus at 6°C/min in each trial. When the mouse licked or shook a hindlimb or jumped, the experimenter immediately removed it from the apparatus and pressed the stop switch on the apparatus to record the surface temperature. Bas experimenter immediately removed it from the apparatus and pressed the stop
switch on the apparatus to record the surface temperature. Baseline threshold was
established on 3 consecutive days, with 3 tests/day. Then, two switch on the apparatus to record the surface temperature. Baseline threshold vestablished on 3 consecutive days, with 3 tests/day. Then, two tests, a non-drug test and a 60 min post-drug, were done daily. Thirty six male established on $\stackrel{\circ}{3}$ consecutive days, with 3 tests/day. Then, two tests, a non-drug pre
test and a 60 min post-drug, were done daily. Thirty six male mice (n=6 per group)
were used. Suvorexant (30 mg/kg in 0.5% methy were used. Suvorexant (30 mg/kg in 0.5% methylcellulose) or vehicle (0.5%
methylcellulose) was given orally 60 min before morphine (5 mg/kg or 10 mg/kg,
subcutaneous). The threshold test started 60 min after morphine. The methylcellulose) was given orally 60 min before morphine (5 mg/kg or 10 m
subcutaneous). The threshold test started 60 min after morphine. The anima
checked for any skin inflammation or lesion from the thermal test and wou subcutaneous). The threshold test started 60 min after morphine. The animal was checked for any skin inflammation or lesion from the thermal test and would have been removed immediately from the experiment for treatment i

Statistical analysis

test. All such tests were two-tailed. Results were considered statistically significant
if P<0.05. The number of subjects in each experimental procedure is indicated by checked for any skin inflammation or lesion from the thermal test and would have
been removed immediately from the experiment for treatment if either occurred,
but this did not happen.
Statistical analysis
Data were subjec been removed immediately from the experiment for treatment if either occurred,
but this did not happen.
Statistical analysis
Data were subjected to ANOVA followed by Tukey post hoc test comparisons or t
test. All such test but this did not happen.

Statistical analysis

Data were subjected to ANOVA followed by Tukey post hoc test comparisons or t

test. All such tests were two-tailed. Results were considered statistically significar

if P<0 But the matter emppyration
Statistical analysis
Data were subjected to *f*
test. All such tests were if P<0.05. The number of
degrees of freedom (df). test. All such tests were two-tailed. Results were considered statistically significal
if P<0.05. The number of subjects in each experimental procedure is indicated by
degrees of freedom (df).
Go if P<0.05. The number of subjects in each experimental procedure is indicated by degrees of freedom (df).
degrees of freedom (df).
Go to degrees of freedom (df).

<u>Go</u> degrees of freedom (df).

Acknowledgements

Go to:

Reference List

1. Cicero T.j. No end in sight. The abuse of prescription narcotics. Cerebrum. cer-11-15
(2015). <u>[PMC free article] [PubMed] [Google Scholar]</u>
2. Mack K.A.. Iones C.M.. & McClure R.I. Physician dispensing of oxycodone and

Support: The Latter of the Alternative and the Department of the Department of Veterans Affairs.

Supported that is a support of the abuse of prescription narcotics. Cerebrum. cer-11-15

(2015). <u>[PMC free article]</u> [PubMe Reference List
1. Cicero T.J. No end
(2015). <u>[PMC free a</u>
2. Mack K.A., Jones (
used opioids, 2000-
article] [<u>PubMed]</u> [<u>(</u>
3. Bass C. & Yates G.
rates of somatoform
(2018). <u>[PubMed]</u> [<u>(</u> (2008). [2016] [PubMed] [PubMed] [PubMed] [PubMed]
2. Mack K.A., Jones C.M., & McClure R.J. Physician dispe
used opioids. 2000-2015. United States. *Pain Med*. 19. used opioids, 2000-2015, United States. *Pain Med*. 19, 990–996 (2018). <u>[PMC free</u> article] [PubMed] [Google Scholar] used opioids, 2000-2015, Onted States. P*um Med*. 19, 990–996 (2010). <u>[EMC free</u>
<u>article] [PubMed] [Google Scholar]</u>
3. Bass C. & Yates G. Complex regional pain syndrome type 1 in the medico-legal se

article] [PubMed] [PubMed]
3. Bass C. & Yates G. Complex region
rates of somatoform disorders. opia rates of somatoform disorders, opiate use and diagnostic uncertainty. Med Sci Law. 58, 147-155
(2018). [PubMed] [Google Scholar] rates of somatoform disorders, opiate use and diagnostic uncertainty. Med Scr Law. 50, 147–155
(2018). <u>[PubMed] [Google Scholar]</u>
4. Kelly M.M.. Reilly E.. Ouinones T.. Desai N.. & Rosenheck R. Long-acting intramuscular

4. Kelly M.M., Reilly E., Quinones T.,
naltrexone for opioid use disorder: naltrexone for opioid use disorder: Utilization and association with multi-morbidity nationally in
the Veterans Health Administration. *Drug Alcohol Depend*.111-117 (2018). <u>[PubMed] [Google</u>
Scholar] the Veterans Health Administration. Drug Alcohol Depend.111-117 (2018). [PubMed] [Google the Veterans Health Administration. *Drug Alcohol Depend.*111–117 (2010). <u>[FubMed] [Google</u>
<u>Scholar]</u>
5. Parthvi R., Agrawal A., Khaniio S., Tsegave A., & Talwar A. Acute opiate overdose: an update (

<u>--------</u>
5. Parthv
managen management strategies in emergency department and critical care unit. Am J Ther. 26, e380–e387
(2019). [PubMed] [Google Scholar] management strategies in emergency department and critical care unit. Am J Ther. 20, e380–e387
(2019). <u>[PubMed] [Google Scholar]</u>
6. McGregor R.. Wu M.-F.. Barber G.. Ramanathan L.. & Siegel I.M. Highly specific role of

(2009). [<u>PubMed] [Boogle Scholars]</u>
6. McGregor R., Wu M.-F., Barber G.,
hypocretin (orexin) neurons: differ hypocretin (orexin) neurons: differential activation as a function of diurnal phase, operant
reinforcement vs. operant avoidance and light level. *Journal of Neuroscience* 31, 15455–154
(2011). [PMC free article] [PubMed] reinforcement vs. operant avoidance and light level. *Journal of Neuroscience* 31, 15455-15467 reinforcement vs. operant avoidance and light level. Journal of Neuroscience 31, 15455–15467
(2011). <u>[PMC free article] [PubMed] [Google Scholar]</u>
7. Wu M.F.. Nienhuis R.. Maidment N.. Lam H.A.. & Siegel I.M. Role of the

7. Wu M.F., Nienhuis R., Maidment N., Lam H.A., & Sieg
receptor 2 (Hcrt-r2) in the regulation of hypocretin le receptor 2 (Hcrt-r2) in the regulation of hypocretin level and cataplexy. *J Neurosci*. 31, 6305–
6310 (2011). [PMC free article] [PubMed] [Google Scholar] receptor 2 (Hcrt-r2) in the regulation of hypocretin level and catapiexy. J Neurosci. 31, 6303–
6310 (2011). <u>[PMC free article] [PubMed] [Google Scholar]</u>
8. Wu M.F.. Nienhuis R.. Maidment N.. Lam H.A.. & Siegel I.M. Cere

8. Wu M.F., Nienhuis R., Maidment N., Lam H.A., & Siegel J.M
631 (orexin) levels are elevated by play but are not raised by ex (orexin) levels are elevated by play but are not raised by exercise and its associated heart rate,
blood pressure, respiration or body temperature changes. Arch. ital. Biol. 149, 492–498
(2011). [PMC free article] [PubMed] blood pressure, respiration or body temperature changes. Arch. *ital. Biol.* 149, 492-498 blood pressure, respiration or body temperature changes. Arch. ital. Biol. 149, 492–498
(2011). <u>[PMC free article] [PubMed] [Google Scholar]</u>
9. Milevkovskiv B.Y.. Kivashchenko L.I.. & Siegel I.M. Behavioral correlates of

(2002).
19. Mileykovskiy B.Y., Kiyashchenko L.I., & Siegel J.M. B
hvpocretin/orexin neurons. *Neuron* 46, 787–798 (200 hypocretin/orexin neurons. Neuron 46, 787-798 (2005). [PMC free article] [PubMed] [Google Scholar] nypocretin/orexin neurons. Neuron 46, 787–798 (2005). <u>[PMC free article] [PubMed] [Google</u>
<u>Scholar]</u>
10. Kivashchenko L.I. et al. Release of hvpocretin (orexin) during waking and sleep states. *I*

<u>– – – – –</u>
10. Kiyas
Neurosci Neurosci 22, 5282-5286 (2002). [PMC free article] [PubMed] [Google Scholar] $Nearost$ 22, 5282–5286 (2002). <u>[PMC free article] [PubMed] [Google Scholar</u>]

11. James M.H., Fragale J.E., O'Connor S.L., Zimmer B.A., & Aston-Jones G. The orexin (hypocretin)
neuropeptide system is a target for novel therapeutics to treat cocaine use disorder with alcohol
co-abuse. *Neuropharmacol* co-abuse. *Neuropharmacology* 108359 (2020). <u>[PMC free article] [PubMed] [Google Scholar]</u>
12. Blouin A.M. et al. Human hypocretin and melanin-concentrating hormone levels are linked to

co-abuse. *Neuropharmacology* 108339 (2020). <u>[PMC free article] [PubMed] [Google Scholar]</u>
12. Blouin A.M. et al. Human hypocretin and melanin-concentrating hormone levels are linke
emotion and social interaction. *Nature* emotion and social interaction. *Nature Communications* 4:1547, 1547 (2013). <u>[PMC free</u>
article] [PubMed] [Google Scholar] emotion and social interaction. Nature Communications 4:1547, 1547 (2015). <u>[PMC free</u>
<u>article] [PubMed] [Google Scholar]</u>
13. Farahimanesh S.. Zarrabian S.. & Haghparast A. Role of orexin receptors in the ventra

article] [PubMed] [PubMed]
13. Farahimanesh S., Zarrabian S., 8
tegmental area on acquisition and e tegmental area on acquisition and expression of morphine-induced conditioned place preference
in the rats. Neuropeptides. 66, 45–51 (2017). [PubMed] [Google Scholar] the rats. *Neuropeptides.* 66, 45–51 (2017). <u>[PubMed] [Google Scholar]</u>
14. Meve F.I.. van Zessen R.. Smidt M.P.. Adan R.A.H.. & Ramakers G.M.I. Morphine withdrawal

in the rats. Neuropeptides. 66, 45–51 (2017). <u>[FubMed] [Google Scholar]</u>
14. Meye F.J., van Zessen R., Smidt M.P., Adan R.A.H., & Ramakers G.M.J. M
enhances constitutive opioid receptor activity in the ventral tegmental a enhances constitutive opioid receptor activity in the ventral tegmental area. *Journal of*
Neuroscience 32, 16120–16128 (2012). [PMC free article] [PubMed] [Google Scholar] enhances constitutive opioid receptor activity in the ventral tegnental area. Journal of
Neuroscience 32, 16120–16128 (2012). <u>[PMC free article] [PubMed] [Google Scholar]</u>
15. Sarti F.. Borgland S.L.. Kharazia V.N.. & Bon

Neuroscience 32, 16120–16128 (2012). <u>[PMC free article] [PubMed] [Google Scholar]</u>
15. Sarti F., Borgland S.L., Kharazia V.N., & Bonci A. Acute cocaine exposure alters spin
and long-term potentiation in the ventral tegmen and long-term potentiation in the ventral tegmental area. *Eur J Neurosci*. 26, 749–756 (2002).
[PubMed] [Google Scholar]

16. Stefano G.B. & Kream R.M. Endogenous morphine synthetic pathway preceded and gave rise. and the set of the Scholars Schol 16. Stefano G.B. [PubMed] [Google Scholar] <u>[PubMed] [Google Scholar]</u>
17. Baimel C. et al. Orexin/hypocretin role in reward: implications for opioid and other

[PubMed] [PubMed]
17. Baimel C. et al. Orexin/h
addictions. *Br I Pharmacol* 1 addictions. *Br J Pharmacol* 172, 334–348 (2015). [<u>PMC free article] [PubMed] [Google S</u>
18. Narita M. et al. Direct involvement of orexinergic systems in the activation of the me

18. Narita M, et al. Direct involvement of orexinergic systems in the activation of the mesolimbic dopamine pathway and related behaviors induced by morphine. *J Neurosci*. 26, 398–405
(2006). [PMC free article] [PubMed] [Google Scholar] dopamine pathway and related behaviors induced by morphine. J Neurosci. 20, 398–405
(2006). <u>[PMC free article] [PubMed] [Google Scholar]</u>
19. Vittoz N.M.. Schmeichel B.. & Berridge C.W. Hypocretin /orexin preferentially a

19. Vittoz N.M., Schmeichel B., & Berridge C.W. Hypocr
caudomedial ventral tegmental area dopamine neuror caudomedial ventral tegmental area dopamine neurons. The European journal of neuroscience 28,
1629–1640 (2008). <u>[PMC free article] [PubMed] [Google Scholar</u>] caudomedial ventral tegmental area dopamme heurons. *The European journal of neuroscience 2*8,
1629–1640 (2008). <u>[PMC free article] [PubMed] [Google Scholar]</u>
20. Pevron C. et al. Neurons containing hvpocretin (orexin) pr

1629–1640 (2009). <u>[PubLe Cooking</u>] [<u>PubLedge Scholar</u>]
1620 (2008). [PubLe Systems. *I Neurosci* 18. 9996–10015 (1998). [PMC free article] [Pu systems. *J Neurosci* 18, 9996-10015 (1998). [PMC free article] [PubMed] [Google Scholar]

21. Guilleminault C. & Cao M.T. Narcolepsy: Diagnosis and management in Principles and Practice *of Sleep Medicine* (eds. Kryger M.H., Roth T. & Dement W.C.) 957–968 (Elsevier Saunders,
Missouri, 2011). [Google Scholar]

22. Galloway G.P. et al. Gamma-hydroxybutyrate: an emerging drug of abuse that causes physical Missouri, 2012).
22. Galloway G.P. et al. Gamma-hy
dependence. Addiction. 92. 89–96 dependence. Addiction. 92, 89–96 (1997). <u>[PubMed] [Google Scholar]</u>
23. Perrotti L.I. et al. Distinct patterns of DeltaFosB induction in brain by drugs of

23. Perrotti L.I. et al. Distinct patterns of DeltaFosB induction in brain by drugs of abuse. Synapse. 62, 358-369 (2008). [PMC free article] [PubMed] [Google Scholar]

24. Zhu J., Spencer T.J., Liu-Chen L.Y., Biederman J., & Bhide P.G. Methylphenidate and opioid receptor interactions: A pharmacological target for prevention of stimulant 23. Zhu J., Spencer T.J., 22. Zhu Biederman J., Biggins P. Methylphenidate and open.
24. The Presention of Stimulant
24. Zhu Development D. (2011). <u>[PMC free article] [PubMed] [Google Schola</u> abuse. *Neuropharmacology* 61, 283–292 (2011). <u>[PMC free article] [PubMed</u>
abuse. Neuropharmacology 61, 283–292 (2011). <u>[PMC free article]</u> [<u>PubMed</u> abuse. Neuropharmacology 61, 203–292 (2011). <u>[PMC free article]</u> [PubMed] [<u>Google Scholar</u>]

25. Darke S., Peacock A., Duflou J., Farrell M., & Lappin J. Characteristics and circumstances of
death related to gamma hydroxybutyrate (GHB). *Clinical Toxicology* 58, 1028–1033 (2020).
[PubMed] [Google Scholar] death related to gamma hydroxybutyrate (GHB). C*linical Toxicology 58, 1028–1033 (2020).*
[<u>PubMed] [Google Scholar]</u>
26. Ponz A. et al. Abnormal activity in reward brain circuits in human narcolepsy with

26. Ponz A. et al. Abnormal
cataplexy. Ann Neurol. 67. 1 cataplexy. *Ann Neurol*. 67, 190–200 (2010). <u>[PubMed] [Google Scholar]</u>
27. Schwartz S. et al. Abnormal activity in hypothalamus and amygdala during humour

catapiexy. Ann Neurol. 67, 190–200 (2010). <u>[FubMed] [Google Scholar]</u>
27. Schwartz S. et al. Abnormal activity in hypothalamus and amygdala
processing in human narcolepsy with cataplexy. *Brain* 131, 514–522 (2 processing in human narcolepsy with cataplexy. *Brain* 131, 514-522 (2007). [PubMed] [Google Scholar] processing in human narcolepsy with cataplexy. *Bruth* 131, 314–322 (2007). <u>[FubMed] [Google</u>
<u>Scholar]</u>
28. Thannickal T.C. et al. Opiates increase the number of hvpocretin-producing cells in mouse ar

<u>--------</u>
28. Than
human b human brain, and reverse cataplexy in a mouse model of narcolepsy. *Sci Transl Med* 10, pii:
eaao4953-doi: 10.1126/scitranslmed.aao4953. (2018). <u>[PMC free article] [PubMed]</u>
[CrossRef] [Google Scholar] eaao4953-doi: 10.1126/scitranslmed.aao4953. (2018). [PMC free article] [PubMed] enable and the control of the matter of the control of the cross Ref] [Google Scholar]
29. Fragale I.E., Iames M.H., & Aston-Iones G. Intermittent self-administration of fenta

[<u>CrossRef]</u>
29. Fragale J.E., James M.H., &
a multifaceted addiction stat a multifaceted addiction state associated with persistent changes in the orexin system. Addict
Biol 2020/August/14, e12946 (2021). [PMC free article] [PubMed] [Google Scholar] a multifaceted addiction state associated with persistent changes in the orexin system. Addict
Biol 2020/August/14, e12946 (2021). <u>[PMC free article] [PubMed] [Google Scholar]</u>
30. Iames M.H. et al. Increased number and a

Biol 2020/August/14, e12946 (2021). <u>[I MC free article] [FubMed] [Google Scholar]</u>
30. James M.H. et al. Increased number and activity of a lateral subpopulation of hyp
orexin/hypocretin neurons underlies the expression o 30. James Prince Prince of all increased in the activity of a lateral subpopulation of an addicted state in rats. *Biol*
Psychiatrydoi: 10.1016/j.biopsych. (2019). <u>[PMC free article] [PubMed] [CrossRef] [Google</u>
Sekelar] orexin/hypocretin neurons underlies the expression of an addicted state in rats. Biol
Psychiatrydoi: 10.1016/j.biopsych. (2019). <u>[PMC free article] [PubMed] [CrossRef] [G</u>
Scholar] r sychiatrydoi: 10.10107j.biopsych. (2019). <u>[PMC free article] [PubMed] [CrossRef] [Google</u>
<u>Scholar]</u>
31. Vickers A.P. Naltrexone and problems in pain management. *BMI* 332, 132–133 (2006). [

<u>– – – – –</u>
31. Vicke
free artic 31. Vickers A.T. Naltrexone and problems in pain management. BMJ 332, 132–133 (2000). <u>[PMC</u>
free article] [PubMed] [Google Scholar]
32. McGregor R. et al. Hvpocretin/orexin interactions with norepinephrine contribute to t

Free articles (Free articles of the school of the scholar
In the school of the scholar scholars of the scholar scholars of the scholar scholars of the scholar scholar opiate withdrawal syndrome. *Journal of Neuroscience* 42, 255–263 (2022). [PMC free article] [PubMed] [Google Scholar] opiate withdrawal syndrome. Journal of Neuroscience 42, 255–263 (2022). <u>[PMC free</u>
<u>article] [PubMed] [Google Scholar]</u>
33. Esmaili-Shahzade-Ali-Akbari P., Hosseinzadeh H., & Mehri S. Effect of suvorexant c

article] [PubMed] [PubMed]
33. Esmaili-Shahzade-Ali-Akbari P.,
tolerance and dependence in mice: tolerance and dependence in mice: Role of NMDA, AMPA, ERK and CREB
proteins. NeuroToxicology 84, 64–72 (2021). [PubMed] [Google Scholar] tolerance and dependence in microbiology 1911 by the USD, and the USD.
The Scholar of Neuro Toxicology 84, 64–72 (2021). <u>[PubMed] [Google Scholar]</u>
34. Gillman A.G., Leffel I.K., Kosobud A.E.K., & Timberlake W. Fentanyl,

proteins. NeuroToxicology 64, 64–72 (2021). <u>[EubMed] [Google Scholar]</u>
34. Gillman A.G., Leffel J.K., Kosobud A.E.K., & Timberlake W. Fentanyl, bu
entrains persisting circadian activity episodes when administered at 24entrains persisting circadian activity episodes when administered at 24- and 31-h intervals. Am J
Psychiatry 2004. Nov. ;161. (11):2126. –8. 2009/07/10, 102-114 (2009). <u>[PMC free</u>
article] [PubMed] [Google Scholar] Psychiatry 2004. Nov.; 161. (11): 2126. -8. 2009/07/10, 102-114 (2009). [PMC free rsychiatry 2004. Nov. ;161. (11):2126. –6. 2009/07/10, 102-114 (2009). <u>[PMC free</u>
<u>article] [PubMed] [Google Scholar]</u>
35. Gillman A.G.. Rebec G.V.. Pecoraro N.C.. & Kosobud A.E.K. Circadian entrainment h

article] [PubMed] [PubMed]
35. Gillman A.G., Rebec G.V., Pecora
drugs of abuse. *Behav Processes* 201 drugs of abuse. Behav Processes 2019/May/24, 23-28 (2019). [PMC free article] [PubMed] [Google Scholar] drugs of abuse. *Behav Trocesses 2019*/May/24, 23–28 (2019). <u>[I MC free</u>
<u>article] [PubMed] [Google Scholar]</u>
36. Iuarez-Portilla C. et al. Brain activity during methamphetamine anticii

article] [PubMed] [PubMed]
36. Juarez-Portilla C. et al. Brain act
self-administration paradigm in mi self-administration paradigm in mice. eNeuro 5, ENEURO (2018). [PMC free article] [PubMed] [Google Scholar] article] $\frac{1}{2}$ [PubMedda] $\frac{1}{2}$ [Google $\frac{1}{2}$ [Google $\frac{1}{2}$]

37. LeSauter J., Balsam P.D., Simpson E.H., & Silver R. Overexpression of striatal D2 receptors
reduces motivation thereby decreasing food anticipatory activity. *The European journal of*
neuroscience 51, 71–81 (2020). [reduces motivation thereby decreasing food anticipatory activity. *The European journal of*
n*euroscience* 51, 71–81 (2020). <u>[PMC free article] [PubMed] [Google Scholar]</u>
38. Correa L.M., Nakai M., Strandgaard C.S., Hess

neuroscience 51, 71–81 (2020). <u>[PMC free article] [PubMed] [Google Scholar]</u>
38. Correa L.M., Nakai M., Strandgaard C.S., Hess R.A., & Miller M.G. Microtubu
testis exhibit differential sensitivity to the microtubule disru testis exhibit differential sensitivity to the microtubule disruptors carbendazim and
colchicine. Toxicological Sciences 69, 175–182 (2002). [PubMed] [Google Scholar] testis exhibit differential sensitivity to the microtubule differential differential secondicine.
The microtological sensitivity of the microtubule disruptors carbinal fluctuation in the number of

colcincine. *Toxicological Sciences 69, 175–162 (2002)*. <u>[FubMed] [Google Scholar]</u>
39. McGregor R., Shan L., Wu M.F., & Siegel J.M. Diurnal fluctuation in the number o
hvpocretin/orexin and histamine producing: Implicati 39. McCream Land And The New York Congress of the number of the number of the hypocretin/orexin and histamine producing: Implication for understanding and treation in the number of the neuronal loss. *PLoS ONE* 12, (2017). hypocretin, or the main that the results of the superior for up the distribution for understanding the sequence
incurrent formal loss. *PLoS ONE* 12, (2017). <u>[PMC free article] [PubMed] [Google Scholar]</u>
40. Thomas T.S.,

40. Thomas T.S., Baimel C., & Borgland S.L. Opioid and hypocretin neuromodulation of ventral tegmental area neuronal subpopulations. *Br J Pharmacol*. 175, 2825–2833 (2018). <u>[PMC free</u>
article] [PubMed] [Google Scholar] tegmental area neuronal subpopulations. Br J Pharmacol. 175, 2025–2033 (2010). <u>[PMC free</u>
<u>article] [PubMed] [Google Scholar]</u>
41. Azizbeigi R.. Farzinnour Z.. & Haghnarast A. Role of orexin-1 recentor within the ventral

<u>article]</u>
41. Azizbeigi R., Farzinpour Z., & Ha
tegmental area in mediating stresstegmental area in mediating stress- and morphine priming-induced reinstatement of conditioned
place preference in rats. *Basic Clin Neurosci*. 10, 373–382 (2019). <u>[PMC free</u>
article] [PubMed] [Google Scholar] place preference in rats. Basic Clin Neurosci. 10, 373-382 (2019). [PMC free place preference in rats. Basic c*lin Neurosci.* 10, 373–302 (2019). <u>[PMC free</u>
<u>article] [PubMed] [Google Scholar]</u>
42. Pantazis C.B., Iames M.H., O'Connor S., Shin N., & Aston-Iones G. Orexin-1

article] [PubMed] [PubMed]
42. Pantazis C.B., James M.H., O'Con
ventral tegmental area mediates cu ventral tegmental area mediates cue-driven demand for
cocaine. Neuropsychopharmacology(2021). [PMC free article] [PubMed] [Google Scholar] ventral tegnental terminalistic cue-driven demand for the area cocaine. Neuropsychopharmacology (2021). [PMC free area 43. Maduna T. et al. Microglia express mu opioid Recepto

cocaine. *Neuropsychopharmacology* (2021). <u>[PMC free article] [PubMed] [Google Scholar]</u>
43. Maduna T. et al. Microglia express mu opioid Receptor: Insights from transcriptomics a
fluorescent reporter mice. *Frontiers in* fluorescent reporter mice. *Frontiers in Psychiatry* 9, (2019). <u>[PMC free article] [PubMed] [Google</u> Scholar] fluorescent reporter intee. Frontiers in Psychiatry 9, (2019). <u>[PMC free article] [PubMed] [Google</u>
<u>Scholar]</u>
44. Horvath R.I. & DeLeo I.A. Morphine enhances microglial migration through modulation of

44. Horv:
P2X4 rec P2X4 receptor signaling. The Journal of Neuroscience 29, 998 (2009). [PMC free
article] [PubMed] [Google Scholar]

45. Machelska H. & Celik M. Opioid receptors in immune and glial cells-implications for pain article] [PubMed] [PubMed]
45. Machelska H. & Celik M. Opioid
control. *Frontiers in Immunology* 11 1944. Machinelska H. & Celik M. Arthur M. O. (PMC free article] [PubMed] [Google Scholar]
16. Cady R.I., Denson I.E., Sullivan L.O., & Durham P.L. Dual orexin receptor antagonist 12 inh

46. Cady R.J., Denson J.E., Sullivan L.Q., & Durham P.L. Dual orexin receptor antagonist 12 inhibits expression of proteins in neurons and glia implicated in peripheral and central
sensitization. *Neuroscience* 269, 79–92 (2014). [PubMed] [Google Scholar] expression of pression of proteins in 1992.
Sensitization, *Neuroscience* 269, 79–92 (2014). <u>[PubMed] [Google Scholar]</u>
47. Li Y. & van den Pol A.N. Mu-opioid recentor-mediated depression of the hvp

sensitization. *Neuroscience 209, 79–92* (2014). <u>[Eublice] [Google Scholar]</u>
47. Li Y. & van den Pol A.N. Mu-opioid receptor-mediated depression of the
hvpocretin/orexin arousal system. *Iournal of Neuroscience* 28. 2814– hypocretin/orexin arousal system. Journal of Neuroscience 28, 2814–2819 (2008). [PMC free
article] [PubMed] [Google Scholar] hypocretin/orexin arousal system. Journal of Neuroscience 20, 2014–2019 (2000). <u>[PMC free</u>
<u>article] [PubMed] [Google Scholar]</u>
48. Stoeber M. et al. A genetically encoded biosensor reveals location bias of opioid drug

<u>article]</u>
48. Stoeber M. et al. A genetically er
action. *Neuron* 98. 963–976 (2018) action. Neuron 98, 963-976 (2018). [PMC free article] [PubMed] [Google Scholar]

49. Peyron C. et al. A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. Nat. Med. . 6, 991–997 (2000).
[PubMed] [Google Scholar] hypocretin peptides in human narcoleptic brains. Nat. Med. . 0, 991–997 (2000).
[PubMed] [Google Scholar] $\frac{1}{2}$ $\frac{1}{2}$

50. Thannickal T.C. et al. Reduced number of hypocretin neurons in human
narcolepsy. *Neuron.* 27, 469–474 (2000). [PMC free article] [PubMed] [Google Scholar]

51. Thannickal T.C. et al. Human narcolepsy is linked to reduced number, size and synaptic bouton density in hypocretin-2 labeled neurons. Abstr Soc Neurosci 26, 2061 (2000). [Google Scholar] bouton density in hypocretin-2 labeled neurons. Abstr Soc Neurosci 26, 2001 (2000). <u>[Google</u>
<u>Scholar]</u>
52. Iames M.H., Mahler S.V., Moorman D.E., & Aston-Iones G. A decade of orexin/hvpocretin ar

<u>– – – – –</u>
52. Jame:
addictior addiction: where are we now? *Curr Top Behav Neurosci* 33, 247–281 (2017). <u>[PMC free</u>
article] [PubMed] [Google Scholar] addiction: where are we now? C*urr Top Behav Neurosci 33, 247–2*01 (2017). <u>[PMC free</u>
<u>article] [PubMed] [Google Scholar]</u>
53. Nestler E.I. Cellular basis of memory for addiction. *Dialogues Clin Neurosci* 15, 431–4

article] [Coordinates] [Coordinates]
53. Nestler E.J. Cellular basis of mer
(2013). [PMC free article] [PubMed (2013). <u>[PMC free article] [PubMed] [Google Scholar]</u>
54. Carrera-Canas C., de Andres I., Callejo M., & Garzon M. Plasticity of the

(2013). [<u>Particle Scholars Scholars J. [PubMed] [PubMed]</u>
54. Carrera-Canas C., de Andres I., Callejo M., & Garzor
hypocretinergic/orexinergic system after a chronic tr For the carrera-canadian, and can alter the Andres II., or and controlled the surface of the process of the process of the Mexicon Mexicon Mexicon Mexicon M. Mexicon M. Mexicon M. Plasticity of the control of the Mexicon M hypocretinergic/orexinergic receptor 1 as an autoreceptor. *Frontiers in Molecular*
Neuroscience 15. (2022). JPMC free article] JPubMed] JGoogle Scholar] nypocretinergic/orexinergic receptor 1 as an autoreceptor. Frontiers in Molecular
Neuroscience 15, (2022). <u>[PMC free article] [PubMed] [Google Scholar]</u>
55. Kaushik M.K. et al. Induction of narcolepsy-like symptoms by ore

Neuroscience 15, (2022). <u>[I MC free article] [I ubMed] [Google Scholar]</u>
55. Kaushik M.K. et al. Induction of narcolepsy-like symptoms by orexin
mice. *Sleep* **DOI:** 10.1093/sleep/zsab043. (2021). [PubMed] [CrossRef] mice. Sleep DOI: 10.1093/sleep/zsab043, (2021). [PubMed] [CrossRef] [Google Scholar]

56. Sora I, et al. Mu opiate receptor gene dose effects on different morphine actions: evidence for differential in vivo mu receptor reserve. Neuropsychopharmacology 25, 41–54 (2001).
[PubMed] [Google Scholar] differential in vivo mu receptor reserve. Neuropsychopharmacology 25, 41–54 (2001).
<u>[PubMed] [Google Scholar]</u>
57. Paqueron X. et al. Is morphine-induced sedation synonymous with analgesia during

[PubMed]
57. Paqueron X. et al. Is mor
intravenous morphine titra intravenous morphine titration? *British Journal of Anaesthesia* 89, 697–701 (2002).
[PubMed] [Google Scholar] intravenous morphine titration: *British Journal of Anaesthesia 69, 697–7*01 (2002).
<u>[PubMed] [Google Scholar]</u>
58. Ito D. et al. Microglia-specific localisation of a novel calcium binding protein. Iba

[<u>PubMed]</u>
58. Ito D. et al. Microglia-sp
Brain Research 57. 1–9 (199 Brain Research 57, 1-9 (1998). [PubMed] [Google Scholar]

Articles from bioRxiv are provided here courtesy of Cold Spring Harbor Laboratory Preprints Articles from bioRxiv are provided here courtesy of Cold Spring Harbor Laboratory Preprints