



OPEN Modulation of dorsal hippocampal SK2 channels regulates morphine-enhanced neuronal oscillations and morphine-induced contextual memory encoding and retrieval

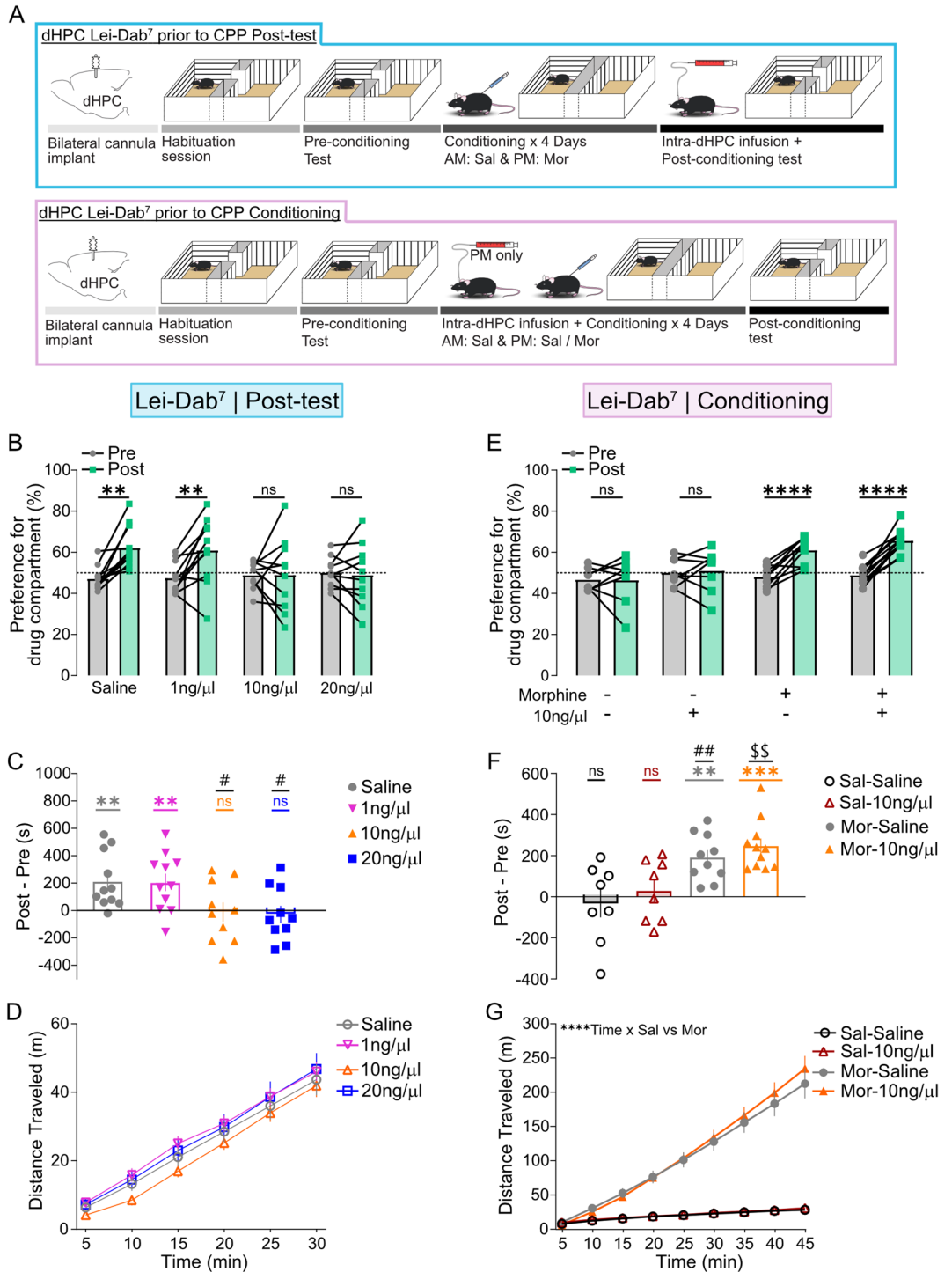
Khairunisa Mohamad Ibrahim^{1,3}, Dominika J. Burek^{1,2,3}, Azra Zec^{1,3}, Olayinka Idowu^{1,3}, Will W. Post^{1,3}, Alice Zheng^{1,3}, Basma Daham^{1,3}, Justin D. Meyer^{1,3}, Sidney Williams^{1,3}, Nicolas Massaly^{1,3} & Jose A. Morón^{1,2,3,4}✉

Persistent maladaptive drug-context associations are a key component of relapse vulnerability and pose a major obstacle in treating opioid use disorder. Although the dorsal hippocampus (dHPC) is critical for contextual memory formation, the synaptic mechanisms by which opioids alter hippocampal circuits to sustain these associations remain unclear. We previously found that repeated context-paired morphine exposure impairs dHPC long-term potentiation (LTP) through enhanced activity of small-conductance calcium-activated potassium (SK2) channels. Here, we investigated the role of SK2 channels in morphine-context associative learning and memory by combining *in vivo* electrophysiology, molecular analyses, and behavioral pharmacology with the morphine-conditioned place preference (MorCPP) paradigm in male mice. Manipulation of SK2 channels with either SK2 blocker Lei-Dab⁷ or activator NS309 before MorCPP post-test dose-dependently reduced MorCPP preference, despite no changes observed in SK2 mRNA or protein expression following MorCPP post-test. Interestingly, SK2 blockade during conditioning instead facilitated morphine-context memory formation, suggesting a distinct SK2 role in drug-context memory acquisition versus retrieval. Electrophysiology data suggest that modulation of dHPC neuronal activity by SK2 channels could be a potential mechanism underlying these observations. Our findings demonstrate that dHPC SK2 channels regulate morphine-context memory formation and retrieval, identifying them as a candidate for mitigating context-driven drug behaviors.

Opioid analgesics are highly effective for acute pain relief, but their use carry the risk of prescription misuse, and the development of opioid use disorders (OUD)¹. OUD is associated with high rates of morbidity and mortality, driven by compulsive drug seeking, withdrawal symptoms, psychiatric comorbidities, and frequent polysubstance use². Persistent maladaptive drug-context associations are thought to contribute to the development and maintenance of OUD, but the mechanisms by which opioid-induced synaptic plasticity creates long-lasting associations between the drug effects and the context in which they are used remain poorly understood^{3–9}.

In preclinical studies, context-dependent locomotor sensitization to morphine (CDLSM) or morphine conditioned place preference (MorCPP) are models that involve drug-context associations. CDLSM measures increased locomotor activity responses following repeated morphine administration in a specific context^{10–14}, while MorCPP measures the learned association between morphine's rewarding properties and contextual environments^{15–19}. We previously demonstrated that context-dependent morphine exposure in both models correlates with increased basal synaptic transmission but impaired long-term potentiation (LTP) in the dorsal hippocampus (dHPC)^{20,21}. Since LTP is a fundamental cellular mechanism for learning and memory, its impairment could prevent the extinction of drug-context associations, potentially facilitating relapse. LTP is dependent on NMDAR activity and mechanistically, we found that CDLSM-induced LTP impairment results

¹Department of Anesthesiology, Washington University in St. Louis, St. Louis, MO 63110, USA. ²Department of Neuroscience, Washington University in St. Louis, St. Louis, MO 63110, USA. ³Washington University in St. Louis Pain Center, St. Louis, MO 63110, USA. ⁴Department of Psychiatry, Washington University in St. Louis, St. Louis, MO 63110, USA. ✉email: jmoron-concepcion@wustl.edu



from an enhanced small-conductance calcium-activated potassium channels type 2 (SK2)-mediated feedback onto NMDARs¹⁰.

SK2 channels have a critical role in the regulation of neuronal excitability and synaptic plasticity through their negative feedback mechanism onto NMDARs. During neuronal activation, calcium influx triggers SK2-mediated potassium efflux, which performs two essential functions: (1) membrane repolarization and (2) restoration of NMDAR magnesium block^{22–24}. This modulation of synaptic activity positions SK2 channels as key regulators of hippocampal-dependent plasticity and learning and memory processes^{24–27}. Importantly, we showed that apamin-induced SK2 blockade restores LTP in CDLSM-sensitized mice¹⁰, suggesting that SK2 activity may be involved in synaptic plasticity necessary for updating maladaptive drug-context associations.

While our previous CDLSM study revealed a correlation between morphine-context exposure and hippocampal LTP impairment, its primary behavioral outcome was sensitized locomotion. To directly test whether SK2-dependent plasticity mechanisms contribute to the rewarding properties of the opioid, we used the MorCPP paradigm, which provides a direct behavioral readout of Pavlovian conditioning related to the drug reward-context associative memory. This approach allowed us to examine how pharmacological modulation

◀ **Fig. 1.** SK2 blocker (Lei-Dab⁷) dose-dependently blocks morphine-context memory retrieval but have no effect on the formation of morphine-context memory with full (4 days) conditioning. (A) Schematic of the experimental timeline for the 4-day conditioning MorCPP protocol. Intra-dHPC saline or Lei-Dab⁷ infusions were administered either before the post-test (light blue) to assess SK2 protein's role in morphine-context memory retrieval or before afternoon conditioning sessions (light pink) to evaluate its role in memory formation. (B) Higher doses of Lei-Dab⁷ (10–20 ng/μl) blocked preference for the morphine-paired compartment, while significant preference was still observed in mice that received the lowest dose of Lei-Dab⁷ (1 ng/μl) or saline in the dHPC ($n = 10–11$; Two-way RM ANOVA, Treatment x Pre vs. Post: $F_{3,38} = 4.557$, $p = 0.0080$ with Sidak's multiple comparisons). (C) Similarly, CPP score (difference in time spent in drug-paired compartment during post-test vs. pre-test) was significantly lower with the higher doses of Lei-Dab⁷ (10–20 ng/μl) compared to the saline group (One-way Anova, $F_{3,38} = 4.226$, $p = 0.0113$ with Dunnett's multiple comparisons; ## vs. Saline). Secondary analysis with one-sample t-test confirmed that only intra-dHPC saline or Lei-Dab⁷ at 1 ng/μl resulted in a significant increase from 0 (indicating no preference) (One-sample t-test, Saline: $t_{10} = 3.557$, $p = 0.0052$, 1 ng/μl: $t_{10} = 3.226$, $p = 0.0091$). (D) Intra-dHPC treatments administered before the post-test did not affect the distance traveled during the post-test, indicating that loss of preference observed with higher doses of Lei-Dab⁷ was not due to impaired locomotion (Two-way RM ANOVA, Treatment x Pre vs. Post: $F_{15,190} = 0.39$, $p = 0.9805$). (E) Intra-dHPC infusion of Lei-Dab⁷ at 10 ng/μl before afternoon conditioning sessions did not block preference for morphine-paired compartment, similar to mice that received intra-dHPC saline infusion. Daily infusion of treatments during conditioning session had no effect on mice behavior as both intra-dHPC saline and Lei-Dab⁷ mice showed no preference for any context when conditioned with saline ($n = 8–11$; Three-way RM ANOVA, Pre vs. Post x Sal vs. Mor: $F_{1,33} = 31.16$, $p < 0.0001$ with Sidak's multiple comparisons). (F) Both morphine-conditioned groups showed a significant increase in CPP score compared to their saline-conditioned counterparts (Two-way ANOVA, Sal vs. Mor: $F_{1,33} = 21.20$, $p < 0.0001$ with Sidak's multiple comparisons within intra-dHPC treatment; ##Mor-Saline vs. Sal-Saline; \$\$Mor-10ng/μl vs. Sal-10ng/μl). Additionally, both morphine-conditioned groups also showed CPP scores significantly greater than 0 (One-sample Wilcoxon test, Mor-Saline: $**p = 0.002$, Mor-10ng/μl: $***p = 0.001$). Although not significant, the higher average CPP score in the Morphine-10 ng/μl group hinted that blocking SK2 channels may facilitate the formation of morphine-context memory (Two-way ANOVA, Sal vs. LeiDab⁷: $F_{1,33} = 1.507$, $p = 0.2283$). (G) Intra-dHPC Lei-Dab⁷ had no effect on locomotion during conditioning, as morphine-conditioned mice exhibited similar distances traveled on conditioning day 1 regardless of the intra-dHPC treatment (Three-way RM ANOVA, Time x Sal vs. Mor: $F_{8,272} = 129.7$, $p < 0.0001$). Data are expressed as mean ± S.E.M. or as mean with before-after pairing.

of SK2 channels (using both blockers and activators) affects the formation and retrieval of morphine-context associations. In addition, we evaluated whether MorCPP alters SK2 expression at the transcriptional or translational level and explored how SK2 blockade relates to hippocampal neuronal activity and theta phase-gamma amplitude coupling during morphine-context re-exposure. Taken together, these experiments advance our understanding of SK2 channel involvement in opioid-associated memory and provide correlative insights into how their activity may influence hippocampal network dynamics underlying the formation and the retrieval of morphine-context associations.

Results

Blocking SK2 activity prevents morphine-context memory retrieval in a dose-dependent manner

Drug-associated contextual memories formed through Pavlovian conditioning play a critical role in OUD vulnerability by eliciting conditioned responses to drug-paired cues. Our previous study demonstrated that pairing morphine with context using the CDLSM paradigm enhanced SK2 channel-mediated negative feedback on NMDAR function in the dHPC¹⁰, implicating dHPC SK2 channel in morphine-related memory processes. However, whether in vivo manipulation of SK2 channel function influences morphine-context memory retrieval has yet to be investigated. To address this gap, we investigated whether SK2 channels in the dHPC are necessary for the retrieval of morphine-context associative memory. Mice were implanted with guide cannulas targeting the dHPC and conditioned with morphine for four days. Immediately before the MorCPP post-test, a selective blocker of SK2 channel activity, Lei-Dab⁷ (1 to 20 ng/μl, 250 nl per side) or vehicle (sterile saline) was infused through cannulas (Fig. 1A; post-test indicated in blue). Morphine-conditioned mice that received intra-dHPC vehicle exhibited significant preference for morphine-paired context (Fig. 1B). In contrast, Lei-Dab⁷ dose-dependently blocked the expression of MorCPP (no preference for morphine-paired context; Fig. 1B). The two highest doses of Lei-Dab⁷ (10 and 20 ng/μl) significantly reduced CPP scores compared to the saline-treated group (Fig. 1C). Further analysis confirmed that CPP scores at these doses were no longer statistically different from a null score of 0, indicating the absence of context preference (Fig. 1C). While LeiDab⁷ prevents the expression of MorCPP, it does not impact animal's locomotor activity, measured by distance traveled, during post-test (Fig. 1D), ruling out nonspecific motor effects. These results build upon our prior work by showing that dHPC SK2 activity is not only involved in modulating NMDAR function after exposure to morphine-context pairing¹⁰ but also crucial for the retrieval of morphine-associated contextual memory.

MorCPP does not alter SK2 gene expression in the hippocampus

While hippocampal activity dynamics can either upregulate or downregulate SK2 expression depending on the isoforms, morphine withdrawal has been shown to increase or decrease SK channel transcript and protein

expression in different brain regions^{28,29}. To understand if the effect of Lei-Dab⁷ on morphine-context associative memory retrieval was possibly due to regulation of SK2 expression, we assessed SK2 gene expression (*KCNN2*) in the hippocampus following MorCPP post-test. Mice were conditioned with either morphine or saline over four days and the whole hippocampus was dissected immediately following the post-test (SFig. 1A). Morphine-conditioned mice exhibited a significant preference for morphine-paired context unlike the saline-conditioned controls (SFig. 1B). Despite their preference for morphine-paired context, no differences in relative *KCNN2* mRNA expression were observed between the morphine- and saline-conditioned (SFig. 1C). Additionally, there was also no significant correlation between the relative *KCNN2* gene expression and the degree of preference for the morphine-paired context among the morphine-conditioned mice (SFig. 1D). These suggest that morphine-context preference is not driven by changes of dHPC SK2 gene expression.

MorCPP does not alter SK2 protein levels in the hippocampus

Given that mRNA transcript expression can account for only up to two-thirds of the variance in protein levels in mice³⁰, it is possible that the SK2 protein levels may be altered with MorCPP even in the absence of changes in mRNA expression. Our previous study found that morphine challenge in the CDLSM paradigm increased SK2 protein in the hippocampal total homogenate fraction but decreased it in the post-synaptic density fraction¹⁰. However, whether MorCPP affects hippocampal SK2 protein levels remains unexplored. To assess this, mice were conditioned with either morphine or saline over four days, and hippocampal tissues were quantified for SK2 channel protein quantification using Western blot (SFig. 1A). As expected, morphine-conditioned mice showed a significant increase in preference for morphine-paired context unlike the controls (SFig. 1E). However, no differences in SK2 protein were observed in total homogenate between saline- and morphine-conditioned mice (SFig. 1F). Additionally, there was no correlation between total homogenate SK2 protein expression and the degree of preference for morphine-paired context (SFig. 1G). Synaptosomal fraction was also analyzed in a subset of the animals and found no morphine-induced changes in SK2 protein levels (SFig. 1H). Similarly, there was no correlation between synaptosomal SK2 protein expression and the degree of preference in morphine-conditioned mice (SFig. 1I). Unlike the CDLSM paradigm¹⁰, these mice did not receive morphine immediately before hippocampal tissue collection, suggesting that changes in SK2 protein levels may require acute morphine presence.

Blocking SK2 activity facilitates the formation of morphine-context memory with subthreshold (2 days) conditioning but not with full (4 days) conditioning

As SK2 channels in the dHPC facilitate the formation of hippocampal-dependent tasks^{25,27}, we also investigated the necessity for SK2 channels to form morphine-context memory in the MorCPP task. Mice were implanted with guide cannulas in the dHPC and underwent a four-day conditioning protocol before the post-test. Immediately before each afternoon conditioning session, mice received intra-dHPC infusions of either Lei-Dab⁷ (10 ng/μl, 250 nl per side) or vehicle. Following infusion, mice were then injected with either saline or morphine (s.c.) and placed in the assigned compartment (Fig. 1A; conditioning indicated in light pink). Treatments remain unchanged throughout the four conditioning days. During post-test, morphine-conditioned mice showed a strong preference for morphine-paired context, unlike the saline-conditioned mice (Fig. 1E). However, intra-dHPC of 10 ng/μl Lei-Dab⁷, a dose that previously blocked morphine-context memory retrieval (Fig. 1B-C), did not affect preference for morphine-paired context when administered during conditioning (Fig. 1E). Daily Lei-Dab⁷ infusions did not affect locomotor activity during conditioning, as morphine-conditioned mice showed a similar increase in locomotor activity regardless of intra-dHPC treatment (Fig. 1G). A two-way ANOVA of the CPP score revealed a significant main effect of drug conditioning (saline vs. morphine). Post-hoc comparisons within each intra-dHPC treatment group showed that morphine-conditioned mice exhibited higher CPP scores than saline-conditioned controls. Specifically, morphine-conditioned mice receiving intra-dHPC Lei-Dab⁷ (Mor-10 ng/μl) had significantly higher CPP score than saline-conditioned mice with intra-dHPC Lei-Dab⁷ (Sal-10 ng/μl), and morphine-conditioned mice with intra-dHPC saline (Mor-Saline) showed greater CPP score than saline-conditioned controls (Sal-Saline). Furthermore, secondary analysis using a one-sample t-test also showed that both morphine-conditioned groups, regardless of intra-dHPC treatment, had CPP scores significantly greater than a null score of 0 (Fig. 1F). The higher CPP score observed in the Mor-10 ng/μl group, although not statistically significant in the two-way ANOVA, may indicate a facilitatory role of morphine-context association, prompting us to test a subthreshold conditioning paradigm. Thus, we used a subthreshold two-day conditioning protocol (Fig. 2A), which did not produce morphine CPP under our experimental conditions, in contrast to the three-day protocol was sufficient to induce CPP (SFig. 2).

Consistent with the protocol test experiments (SFig. 2E-F), morphine-conditioned mice that received intra-dHPC saline (vehicle) did not exhibit a significant increase in preference for morphine-paired context (Fig. 2B). In contrast, morphine-conditioned mice that received intra-dHPC Lei-Dab⁷ (10 ng/μl) developed a clear preference for morphine-paired context (Fig. 2B). A two-way ANOVA of the CPP score revealed a significant main effect of drug conditioning (saline vs. morphine). Post-hoc comparisons within each intra-dHPC treatment group showed that while morphine-conditioned mice with intra-dHPC saline (Mor-Saline) did not differ from saline controls (Sal-Saline), morphine-conditioned mice with intra-dHPC Lei-Dab⁷ (Mor-10 ng/μl) showed significantly higher CPP score than their saline-conditioned counterparts (Sal-10 ng/μl). This indicates that the main effect was primarily driven by the Mor-10 ng/μl group. In addition, secondary analysis using a one-sample t-test also showed that only morphine-conditioned mice with intra-dHPC Lei-Dab⁷ had CPP scores significantly greater than a null score of 0 (Fig. 2C). Saline-conditioned mice, regardless of intra-dHPC treatment, did not exhibit preferences for any compartments or show changes in CPP score (Fig. 2B-C). These findings suggest that blocking dHPC SK2 channels facilitates the formation of morphine-context memory, as fewer conditionings were required to establish preference for morphine-paired context. Similarly, two consecutive days of intra-

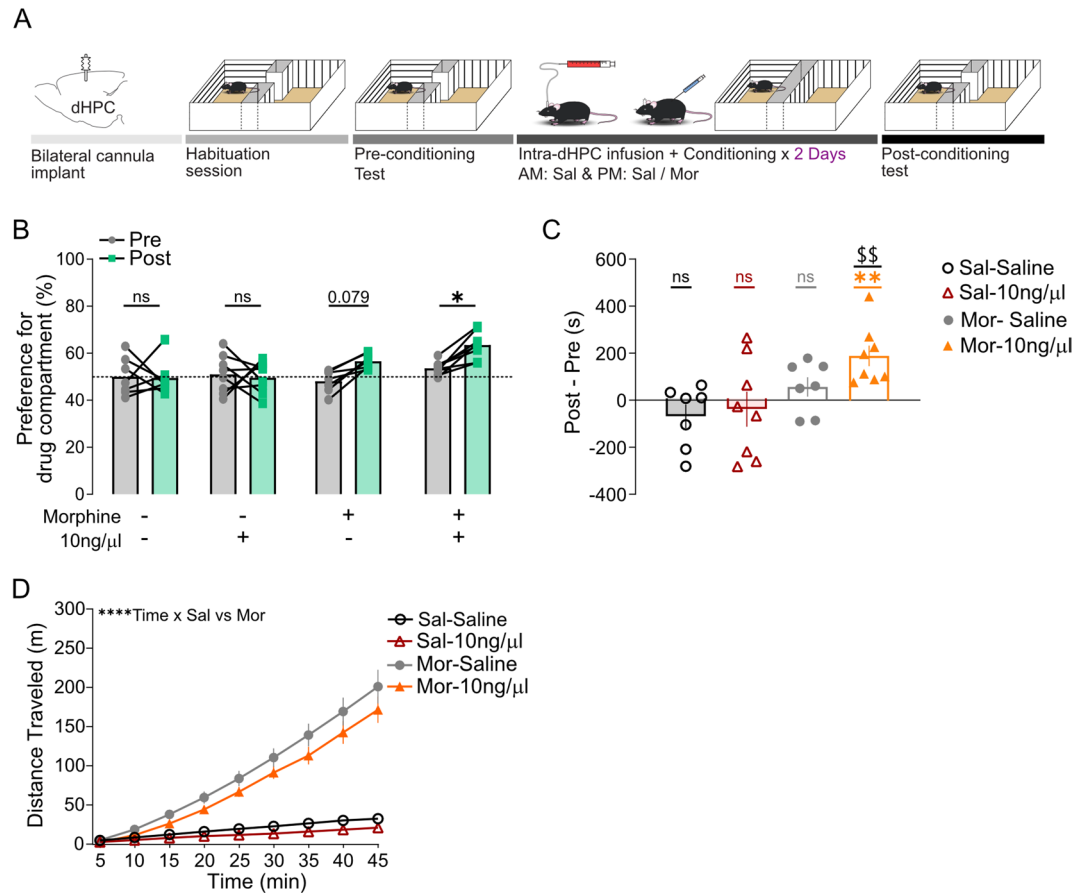
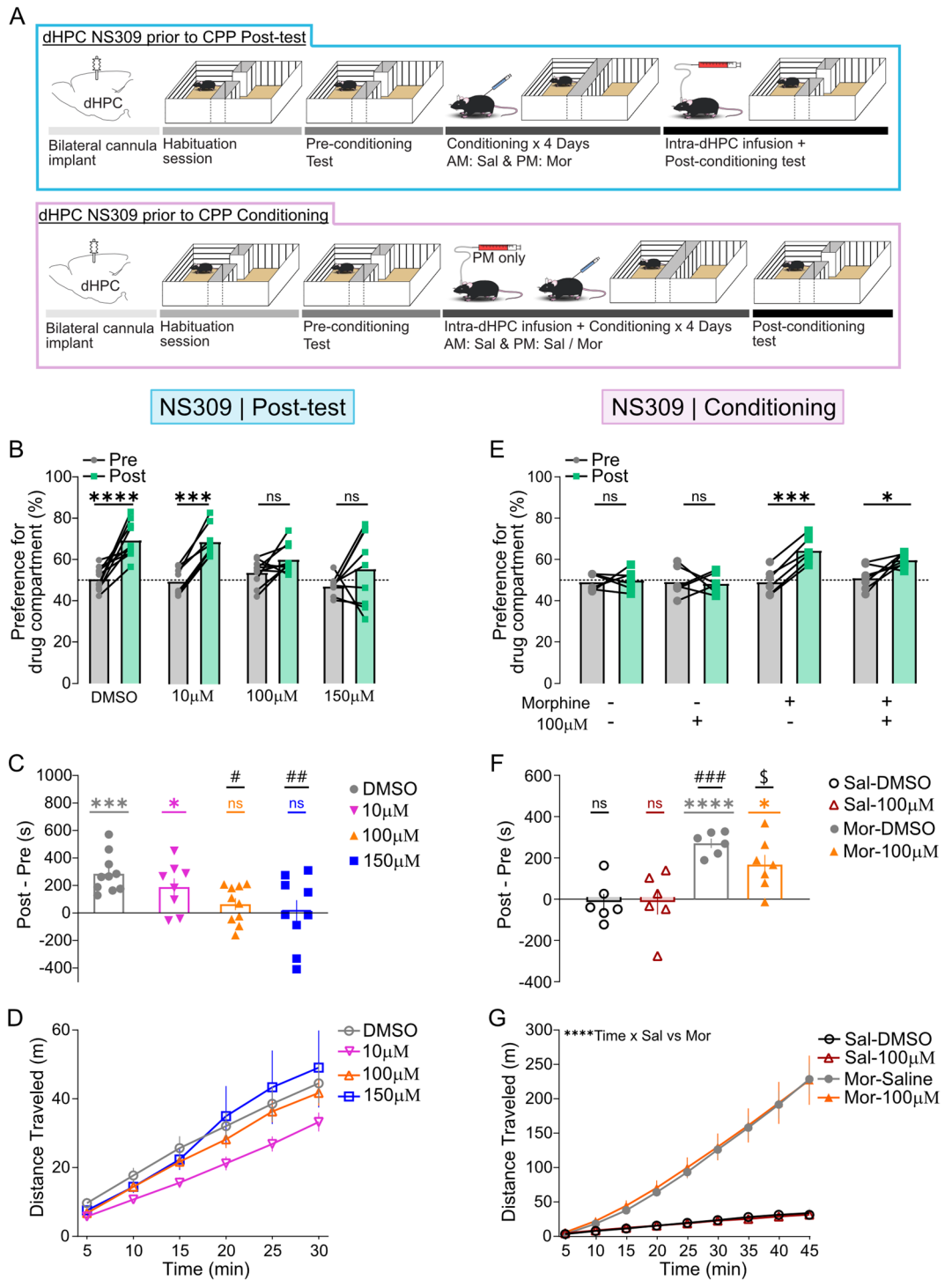


Fig. 2. Intra-dHPC Lei-Dab⁷ facilitates the formation of morphine-context memory with subthreshold (2 days) conditioning. **(A)** Schematic of the experimental timeline for the subthreshold 2-day conditioning MorCPP protocol. Intra-dHPC saline or Lei-Dab 7 infusions were administered before afternoon conditioning sessions to evaluate SK2 channel's role in memory formation. **(B)** Two days of morphine conditioning were insufficient to induce a significant preference for the morphine-paired compartment. However, mice that received Lei-Dab⁷ (10 ng/ μ l) prior to conditioning sessions showed a strong preference for the morphine-paired compartment, suggesting that blocking SK2 channels facilitates the formation of morphine-context memory, as fewer conditioning sessions were required to induce significant preference ($n = 7-8$; Three-way RM ANOVA, Pre vs. Post \times Sal vs. Mor: $F_{1,26} = 9.274$, $p = 0.0053$ with Sidak's multiple comparisons). **(C)** Similarly, only morphine-conditioned mice that received intra-dHPC Lei-Dab⁷ showed a significant increase in CPP score compared to its saline-conditioned counterpart (Two-way RM ANOVA, Sal vs. Mor: $F_{1,26} = 15.62$, $p = 0.0005$ with Sidak's multiple comparisons; $\$$ Mor-10ng/ μ l vs. Sal-10ng/ μ l) and from 0 (One-sample t-test, Mor-LeiDab: $t_7 = 4.345$, $**p = 0.0034$). **(D)** Intra-dHPC Lei-Dab 7 had no effect on locomotion during conditioning, as morphine-conditioned mice exhibited similar distances traveled on conditioning day 1 regardless of the intra-dHPC treatment (Three-way RM ANOVA, Time \times Sal vs. Mor: $F_{8,208} = 121.5$, $p < 0.0001$). Data are expressed as mean \pm S.E.M. or as mean with before-after pairing.

dHPC infusions did not affect locomotor activity during conditioning (Fig. 2D). In summary, SK2 channels are necessary for retrieval of morphine-context memory, but their blockage during conditioning instead facilitated morphine-context formation.

SK2 activation in the dHPC prevents morphine-context memory retrieval

To determine whether activating SK2 channels disrupts morphine-context memory formation or retrieval, separate cohorts of mice were infused with the SK2 activator, NS309, either before post-test or afternoon conditioning sessions (Fig. 3A). Administration of NS309 is shown enhance SK2-mediated afterhyperpolarization currents, leading to reduced intrinsic excitability of dHPC pyramidal cells and impairment of hippocampus-dependent learning tasks^{22,24}. Morphine-conditioned mice that received intra-dHPC infusions of either 0.7% DMSO (vehicle) or the lowest dose of NS309 (10 μ M) before the post-test exhibited a significant preference for the morphine-paired context and higher CPP score (Fig. 3B-C). In contrast, intra-dHPC of higher doses of NS309 (100–150 μ M) in morphine-conditioned mice blocked preference for morphine-paired context (Fig. 3B) and a decrease in CPP score (Fig. 3C). The effect of NS309 on MorCPP behaviors was not attributed to changes in locomotion activity as all groups exhibited similar distances traveled during the post-test (Fig. 3D). This suggests that activation of dHPC SK2 channels with NS309 blocks the retrieval of morphine-context memory.



However, morphine-conditioned mice that received daily intra-dHPC infusions of NS309 (100 μ M) prior to conditioning still exhibited a significant preference for the morphine-paired context (Fig. 3E). A two-way ANOVA of the CPP score revealed a significant main effect of drug conditioning (saline vs. morphine). Post-hoc comparisons within each intra-dHPC treatment group showed that morphine-conditioned mice exhibited higher CPP scores than saline-conditioned controls. Specifically, morphine-conditioned mice receiving intra-dHPC NS309 (Mor-100 μ M) had significantly higher CPP score than saline-conditioned mice with intra-dHPC NS309 (Sal-100 μ M), and morphine-conditioned mice with intra-dHPC saline (Mor-DMSO) showed greater CPP score than saline-conditioned controls (Sal-DMSO). Furthermore, secondary analysis using a one-sample t-test also showed that both morphine-conditioned groups, regardless of intra-dHPC treatment, had CPP scores significantly greater than a null score of 0 (Fig. 3F). This indicates that activating SK2 channels with NS309 during conditioning had minimal impact on the formation of morphine-context memory. Daily infusion of NS309 or DMSO prior to conditioning also had no effect on morphine-induced locomotor activity (Fig. 3G). Saline-conditioned mice, regardless of intra-dHPC treatment, did not exhibit preferences for any compartments

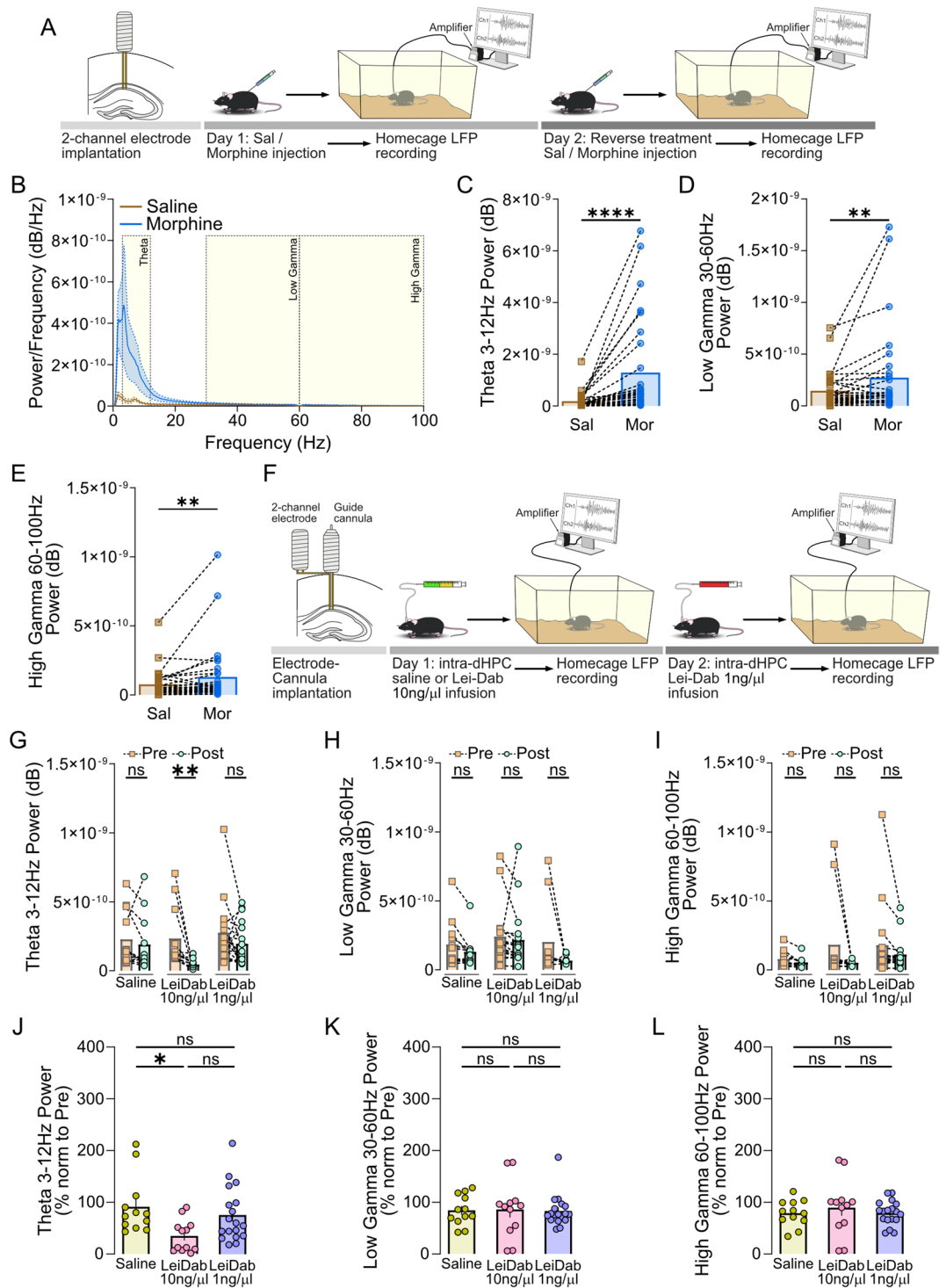
◀ **Fig. 3.** SK2 activator (NS309) dose-dependently blocks morphine-context memory retrieval. **(A)** Schematic of the experimental timeline for the 4-day conditioning MorCPP protocol. Intra-dHPC DMSO (vehicle) or NS309 infusions were administered either before the post-test (light blue) to assess SK2 protein's role in morphine-context memory retrieval or before afternoon conditioning sessions (light pink) to evaluate its role in memory formation. **(B)** Higher doses of NS309 (100–150 μM) blocked preference for the morphine-paired compartment, while significant preference was still observed in mice that received the lowest dose of NS309 (10 μM) or DMSO in the dHPC ($n=8-10$; Two-way RM ANOVA, Treatment x Pre vs. Post: $F_{3,33} = 2.908$, $p=0.0491$ with Sidak's multiple comparisons). **(C)** Similarly, CPP score (difference in time spent in drug-paired compartment during post-test vs. pre-test) was significantly lower with the higher doses of NS309 (100–150 μM) compared to the saline group (One-way Anova, $F_{3,33} = 4.471$, $p=0.0097$ with Dunnett's multiple comparisons; ## vs. DMSO). Secondary analysis with one-sample t-test confirmed that only intra-dHPC DMSO or NS309 at 10 μM resulted in a significant increase from 0 (One-sample t-test, DMSO: $t_9 = 4.657$, $***p=0.0012$, 10 μM : $t_9 = 3.016$, $*p=0.0195$). **(D)** Intra-dHPC treatments administered before the post-test did not affect the distance traveled during the post-test, indicating that loss of preference observed with higher doses of NS309 was not due to impaired locomotion (Two-way RM ANOVA, Treatment x Pre vs. Post: $F_{15,165} = 0.8543$, $p=0.6161$). **(E)** Intra-dHPC infusion of NS309 at 100 μM before afternoon conditioning sessions did not block preference for morphine-paired compartment, similar to mice that received intra-dHPC DMSO infusion. Daily infusion of treatments during conditioning session had no effect on mice behavior as both intra-dHPC DMSO and NS309 mice showed no preference for any context when conditioned with saline ($n=6-7$; Three-way RM ANOVA, Pre vs. Post x Sal vs. Mor: $F_{1,21} = 15.65$, $p=0.0007$ with Sidak's multiple comparisons). **(F)** Both morphine-conditioned groups showed a significant increase in CPP score compared to saline-conditioned groups (Two-way RM ANOVA, Sal vs. Mor: $F_{1,21} = 26.80$, $p<0.0001$ with Sidak's multiple comparisons; ###Mor-DMSO vs. Sal-DMSO; \$Mor-100 μM vs. Sal-100 μM) and from 0 (One-sample t-test, Mor-DMSO: $t_5 = 11.85$, $****p<0.0001$, *Mor-NS309: $t_6 = 3.568$, $p=0.0118$). **(G)** Intra-dHPC NS309 did not affect locomotion during conditioning, as morphine-conditioned mice exhibited similar distances traveled on conditioning day 1 regardless of the intra-dHPC treatment (Three-way RM ANOVA, Time x Sal vs. Mor: $F_{8,168} = 63.93$, $p<0.0001$). Data are expressed as mean \pm S.E.M. or as mean with before-after pairing.

or show changes in CPP score (Fig. 3E-F). Overall, blocking SK2 channels with Lei-Dab⁷ dose-dependently impaired morphine-context memory retrieval but facilitates its formation under subthreshold conditioning, whereas activating SK2 channels with NS309 dose-dependently impaired morphine-context memory retrieval with minimal impact on morphine-context formation.

Morphine enhances dHPC neuronal oscillations, whereas under drug-naïve conditions intra-dHPC SK2 Blockade reduces them

After establishing that SK2 channel activity in the dHPC is necessary for the retrieval of morphine-context associative memory, we sought to identify the potential mechanism underlying these behavioral effects. Neuronal population dynamics in the dHPC, particularly theta and gamma oscillations, play a critical role in encoding learning and memory processes. These neuronal oscillations have been strongly implicated in formation, consolidation, and retrieval of reward-related learning tasks, reflecting the underlying mechanisms of information processing during such behaviors³¹⁻³³. To dissect the role of SK2 channels on morphine-context memory, we first characterized how acute morphine (Fig. 4A-E) and SK2 inhibition (Fig. 4F-I) alter dHPC neuronal activity. To assess the effect of an acute morphine injection on dHPC neuronal activity, mice were subcutaneously injected with either saline or morphine before the start of LFP recording in a home cage setting (Fig. 4A). Morphine administration resulted in a significant increase in overall power spectral density compared to saline (Fig. 4B). Specifically, morphine induced a significant increase in the total power of the theta (Fig. 4C), low gamma (Fig. 4D), and high gamma frequency bands (Fig. 4E). Because oscillatory power alone does not fully capture the complexity of hippocampal network computations, we next examined phase-amplitude coupling (PAC), a cross-frequency interaction in which the phase of slower theta rhythms modulates the amplitude of faster gamma oscillations. PAC has been proposed to facilitate temporal coordination of neuronal ensembles, thereby supporting synaptic plasticity and memory encoding and retrieval³⁴⁻³⁷. Despite the increase in theta and gamma power following morphine administration, the distribution of low- and high-gamma amplitude across theta phases was comparable after saline and morphine administration (SFig. 3A, C). No significant differences were also observed in normalized modulation index (MI) values for either low or high gamma frequency bands (SFig. 3B, D).

To investigate the role of SK2 channels in modulating dHPC LFP activity, an electrode-cannula combo was implanted in the dHPC, enabling infusion of SK2 blocker, Lei-Dab⁷, and LFP recordings in the same dHPC region (Fig. 4F). Infusion of the higher Lei-Dab⁷ concentration (10 ng/ μl) produced a significant reduction in theta power relative to pre-infusion (Fig. 4G), whereas low- and high-gamma power were not affected (Fig. 4H-I). Consistently, the normalized post-infusion theta power was significantly decreased with 10 ng/ μl Lei-Dab⁷ compared to saline, but not relative to 1 ng/ μl Lei-Dab⁷. No differences between treatment groups were observed with low- or high-gamma power (Fig. 4K-L). Similar to morphine, the distribution of low- and high-gamma amplitude across theta phases was comparable across treatment during pre- and post-infusion recordings (SFig. 3E-F, H-I). Similarly, a two-way RM ANOVA analysis showed no significant differences in the normalized MI values for either gamma bands between treatment groups or across pre- vs. post-infusions conditions (SFig. 3G, J). Overall, these findings revealed that hippocampal neuronal activity is involved in processing morphine reward, while SK2 inhibition reduces drug-naïve dHPC activity.



Blocking SK2 activity in the dHPC prevents LFP enhancement associated with morphine-context re-exposure

To investigate whether SK2 channels modulate neuronal oscillations during morphine-context re-exposure, LFP recordings were conducted in two distinct contexts before and after conditioning (each context was paired with either saline or morphine). The same MorCPP apparatus and post-test dHPC infusion protocol were used as in our previous experiments (Fig. 5A). In control animals receiving intra-dHPC saline, re-exposure to the morphine-paired context produced the expected significant increase in theta and gamma frequency bands (Fig. 5B-D). In contrast, following intra-dHPC administration of the SK2 channel blocker Lei-Dab⁷, re-exposure to the morphine-paired context no longer elicited increases in theta or gamma power (Fig. 5E-G). Re-exposure to the saline-paired context did not alter LFP activity in either control or Lei-Dab⁷-treated mice (Fig. 5B-G). Similarly, direct comparison of post-test recordings showed oscillatory power was significantly elevated upon re-exposure to the morphine-paired context in intra-dHPC saline mice, but not in Lei-Dab⁷-treated mice (Fig. 5H-J). Importantly, these effects were not attributable to differences in locomotor activity. Distance traveled was

◀ **Fig. 4.** Morphine enhances dHPC neuronal oscillations, while intra-dHPC SK2 blockade reduces them under drug-naïve conditions. **(A)** Schematic outlining the experimental timeline of dHPC local field potential (LFP) recording with morphine administration in the home cage. After recovery from electrode implantation, mice received either morphine (s.c, 15 mg/kg) or saline (sal, s.c) before being connected to the amplifier and placed back into the home cage. dHPC LFP was recorded for 30 min. The experiment was repeated after 24 h with the exception that the treatment (morphine or sal) was reversed ($n = 30$ electrodes). **(B)** Morphine administration (s.c, 15 mg/kg) in the home cage induced an increase in power spectral density (PSD) across the 0–100 Hz frequency band compared to saline injections. Specifically, morphine administration in the home cage induced a significant increase in total power in **(C)** theta (3–12 Hz) frequency band (Wilcoxon matched-pairs signed rank test, $p < 0.0001$), **(D)** low gamma (30–60 Hz) frequency band (Wilcoxon matched-pairs signed rank test, $p = 0.0012$) and **(E)** high gamma (60–100 Hz) frequency band (Wilcoxon matched-pairs signed rank test, $p = 0.0024$). **(F)** Schematic outlining the experimental timeline of dHPC local field potential (LFP) recording with intra-dHPC infusion of either saline or Lei-Dab⁷ (1 or 10 ng/μl; 250 nl) in the home cage. After recovery from electrode implantation, mice received either intra-dHPC saline or Lei-Dab⁷ at 10 ng/μl before being connected to the amplifier and placed back into the home cage. dHPC LFP was recorded for 30 min. The experiment was repeated after 24 h with all animals receiving intra-dHPC Lei-Dab⁷ at 1 ng/μl before the start of the experiment ($n = 12$ –18 electrodes). **(G)** Intra-dHPC infusion of Lei-Dab⁷ at 10 ng/μl in the home cage induced a significant decrease in total power for theta (3–12 Hz) frequency band from pre-infusion (Two-way RM ANOVA, Pre vs. Post: $F_{1,39} = 10.68$, $p = 0.0023$ with Sidak's multiple comparisons). Intra-dHPC infusion of Lei-Dab⁷ at 10 ng/μl in the home cage had no effect on total power for **(H)** low gamma (30–60 Hz) frequency band (Two-way RM ANOVA, Pre vs. Post: $F_{1,39} = 4.437$, $p = 0.0417$ with Sidak's multiple comparisons) and **(I)** high gamma (60–100 Hz) frequency band (Two-way RM ANOVA, Pre vs. Post: $F_{1,39} = 5.884$, $p = 0.02$ with Sidak's multiple comparisons). **(J)** Normalized theta power (post-test power/pre-test power $\times 100$) was significantly reduced following intra-dHPC infusion of Lei-Dab⁷ at 10 ng/μl compared to saline group but not relative Lei-Dab⁷ at 1 ng/μl (Kruskal-Wallis test; $H_3 = 9.099$, $p = 0.0106$ with Dunn's multiple comparisons). **(K)** Normalized low gamma and **(L)** high gamma power showed no significant differences across treatment groups (Low gamma: Kruskal-Wallis test; $H_3 = 0.1809$, $p = 0.9135$; High gamma: One-way ANOVA, $F_{2,39} = 0.3875$, $p = 0.6813$). Data are expressed as mean \pm S.E.M. or as mean with before-after pairing.

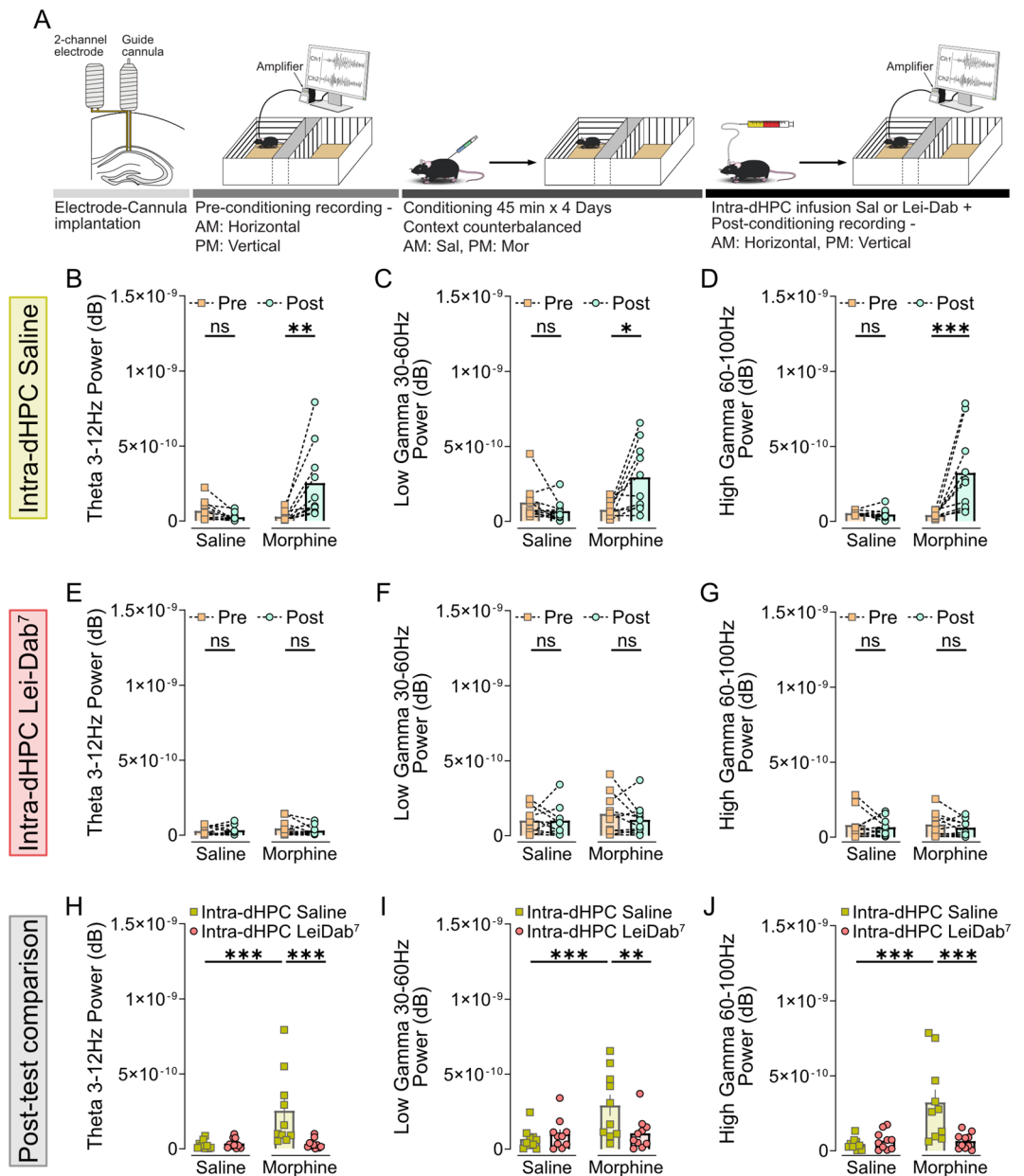
comparable across treatment groups both before conditioning (pre-test) and during re-exposure (post-test) (SFig. 4A–B). Furthermore, no significant correlation was found between hippocampal theta power and total distance traveled during the post-test session (SFig. 4C–D).

We next examined whether SK2 channels also modulate phase–amplitude coupling (PAC) during morphine-context re-exposure. Intra-dHPC saline mice showed similar distributions of low- and high-gamma amplitude across theta phases during pre- and post-saline compartments (SFig. 5A, D). However, re-exposure to the morphine-paired compartment produced a shift in both low- and high-gamma amplitude distributions relative to pre-morphine context exposure (SFig. 5B, E). This is accompanied by a significant increase in normalized MI for low gamma compared to both the pre-morphine compartment and the post-saline compartment (SFig. 5C). By contrast, the shift in amplitude distribution (SFig. 5H, K) and the increase in low gamma normalized MI (SFig. 5I) upon re-exposure to morphine-paired context were not observed in the intra-dHPC Lei-Dab⁷ group. Intra-dHPC Lei-Dab⁷ mice also showed similar distributions of low- and high-gamma amplitude across theta phases during pre- and post-saline compartments (SFig. 5G, J). Normalized MI for high gamma is similar across saline- and morphine-paired compartments and across pre- and post-exposure in both saline and Lei-Dab⁷ groups (SFig. 5F, L). Taken together, these findings indicate that blocking SK2 channels coincides with the absence of increased dHPC oscillatory power and theta phase-low gamma coupling that is triggered by re-exposure to a morphine-paired context, suggesting a potential role for SK2 channels in modulating hippocampal network dynamics underlying morphine-context association.

Discussion

The present study builds on our previous work¹⁰ providing significant insights into the role of dHPC SK2 channels in the retrieval and formation of morphine-context associative memory. Both blocking dHPC SK2 channel with Lei-Dab⁷ (Fig. 1B–D) or activating it with NS309 (Fig. 3B–D) dose-dependently impaired the retrieval of morphine-context memory without affecting locomotor activity, suggesting the need for a balanced SK2 channel activity for successful recall of morphine-associated context. While blocking SK2 channels had no effect on morphine-context memory formation during full (4-day) conditioning (Fig. 1E–G), it facilitated learning under subthreshold (2-day) conditioning (Fig. 2). Electrophysiological recordings revealed that subcutaneous morphine (Fig. 4B–E) and re-exposure to morphine-paired context (Fig. 5) enhanced dHPC theta and gamma power, while SK2 inhibition suppressed the increase in power during morphine-context re-exposure. Re-exposure to the morphine-paired context also increased theta phase-low gamma amplitude coupling, which was not observed in SK2-treated mice (SFig. 5), suggesting that SK2 channels influence hippocampal oscillatory dynamics accompanying morphine-context retrieval.

Hippocampal theta oscillations reflect a state of readiness for information processing^{38–41}, while gamma oscillations are primarily linked to memory formation^{39,42}. Their interaction through phase–amplitude coupling (PAC) coordinates hippocampal ensembles during encoding and retrieval^{34,35}. Subcutaneous morphine in the home cage increased both theta and gamma oscillations (Fig. 4A–E), indicating activation of hippocampal networks during morphine exposure. Although morphine-induced hyperlocomotion^{43,44} may partly explain increased theta power^{45–47}, during re-exposure to the morphine-paired context, the increase in theta power



was not accompanied by an increase in locomotion (SFig. 4). This suggests that the oscillatory changes during memory retrieval cannot be fully explained by locomotor activity and may reflect context-driven neural processes. PAC analysis further showed that acute morphine increased oscillatory power without altering coupling (SFig. 3) but morphine-paired context re-exposure selectively increased theta–low gamma coupling (SFig. 5). This dissociation suggests that changes in oscillatory power may reflect general network activation, whereas coupling changes relate more specifically to memory retrieval.

SK2 inhibition in the home cage reduced theta but not gamma power (Fig. 4F–I). In contrast, it suppressed both oscillatory increases and enhanced coupling during morphine-context re-exposure (Fig. 5, SFig. 5), suggesting a role for SK2 in coordinating neuronal dynamics required for retrieving morphine-context associations. As SK2 inhibition impairs PAC in morphine-associated context and not in the home cage, SK2 channels appear to be specifically involved in associating the context with morphine, consistent with prior evidence that SK2 changes arise only when morphine is paired with a novel context¹⁰.

As these LFP experiments were carried out separately from the MorCPP behavior experiments, they reflect a correlative SK2-dependent network dynamic that parallels SK2 effects on MorCPP behaviors. However, the selective increase in theta–low-gamma coupling during morphine-context retrieval suggests that SK2-sensitive dynamics are engaged for the retrieval of a salient drug-context association, rather than supporting a general contextual memory mechanism that would be required for both morphine and saline contexts. Nevertheless, future studies combining oscillatory manipulation with SK2 modulations of MorCPP will be needed to test causality.

Despite significant behavioral and electrophysiological effects, no changes in either SK2 gene or protein expression were detected in the hippocampus following MorCPP post-test (SFig. 1). While this finding aligns

◀ **Fig. 5.** Intra-dHPC Lei-Dab⁷ blocked the increase in dHPC neuronal activity with re-exposure to morphine-paired context. **(A)** Schematic outlining the experimental timeline of dHPC LFP recording during morphine-context exposure. After recovery from electrode implantation, dHPC LFP was recorded as mice explored two distinct contexts (morning: horizontal, afternoon: vertical) using the same MorCPP apparatus as previous experiments. During conditioning, these contexts were paired with either morphine (s.c, 15 mg/kg) or saline (sal, s.c) in a counterbalanced manner. 24 h after the last conditioning day, mice received intra-dHPC infusions of either saline or Lei-Dab⁷ (10 ng/μl; 250nl) before being placed in the contexts (morning: horizontal, afternoon: vertical) while dHPC LFP was recorded ($n = 10$ electrodes). Mice that received intra-dHPC saline infusion showed a significant increase in **(B)** theta frequency band power (Two-way RM ANOVA, Treatment x Pre vs. Post: $F_{1,9} = 14.48, p = 0.0042$ with Sidak's multiple comparisons; Morphine Pre vs. Post: $p = 0.0011$), **(C)** low gamma frequency band power (Two-way RM ANOVA, Treatment x Pre vs. Post: $F_{1,9} = 8.198, p = 0.0187$ with Sidak's multiple comparisons; Morphine Pre vs. Post: $p = 0.0021$) and **(D)** high gamma frequency band power (Two-way RM ANOVA, Treatment x Pre vs. Post: $F_{1,9} = 11.49, p = 0.0080$ with Sidak's multiple comparisons; Morphine Pre vs. Post: $p = 0.0004$) during post-conditioning compared to pre-conditioning test. Exposure to saline-paired context showed no increase in the total power of any of the frequency bands. In contrast, intra-dHPC Lei-Dab⁷ infusion blocked any morphine-context-induced increase in total power at **(E)** theta frequency band power (Two-way RM ANOVA, Treatment x Pre vs. Post: $F_{1,9} = 1.266, p = 0.2896$), **(F)** low gamma frequency band power (Two-way RM ANOVA, Treatment x Pre vs. Post: $F_{1,9} = 1.599, p = 0.2379$) and **(G)** high gamma frequency band power (Two-way RM ANOVA, Treatment x Pre vs. Post: $F_{1,9} = 0.08675, p = 0.7750$). Comparison of post-test dHPC LFP recording showed that re-exposure to the morphine-paired context significantly increased power in **(H)** theta frequency band power (Two-way RM ANOVA, Sal vs. Mor x Sal vs. LeiDab: $F_{1,18} = 9.310, p = 0.0069$), **(I)** low gamma frequency band power (Two-way RM ANOVA, Sal vs. Mor x Sal vs. LeiDab: $F_{1,18} = 9.096, p = 0.0074$) and **(J)** high gamma frequency band power (Two-way RM ANOVA, Sal vs. Mor x Sal vs. LeiDab: $F_{1,18} = 11.32, p = 0.0035$) in mice receiving intra-dHPC saline. This increase in oscillatory power was no longer observed in mice that received intra-dHPC Lei-Dab⁷. Data are expressed as mean \pm S.E.M. or as mean with before-after pairing.

with previous reports showing unchanged SK2 protein expression after contextual fear memory²⁷, it differs from our previous CDLSM study¹⁰, likely due to the absence of a morphine challenge before dHPC tissue collection in the current work. Given that CA1 SK2 expression is restored by 2 h after long-term potentiation (LTP) induction⁴⁸, SK2 expression changes may be transient, supporting the idea that MorCPP expression is driven by increased SK2 function rather than lasting protein upregulation.

This is supported by our data demonstrating that blocking SK2 channels disrupts MorCPP expression in a dose-dependent manner (Fig. 1B & C). Furthermore, intra-dHPC Lei-Dab⁷ infusion prevented the increase in LFP induced by the re-exposure to morphine-paired context (Fig. 5), supporting that dHPC SK2 channels' activity contributes to MorCPP expression. Interestingly, activating dHPC SK2 channels with NS309 also blocked the expression of MorCPP in a dose-dependent manner (Fig. 3B & C). Although the effect of NS309 on dHPC LFP during morphine-paired context exposure remains to be investigated, SK2 channel activation enhances the amplitude and duration of afterhyperpolarizing (AHP) currents that follow action potentials^{22,49}. SK2 channels are activated by an increase in intracellular calcium concentration, and in turn increase potassium efflux which leads to neuronal hyperpolarization, limiting further firing⁵⁰. By regulating neuronal excitability, SK2 channels influence the dHPC network activity that supports memory retrieval. Disrupting normal SK2 function either through blockade or activation alters neuronal excitability in ways that may prevent the formation of appropriate theta and gamma oscillatory patterns. This could explain why both manipulations impaired retrieval of morphine-context memories, albeit through opposing mechanisms: SK2 blockade increases excitability while activation reduces it, with both conditions potentially disrupting the theta/gamma coordination required for MorCPP expression.

Beyond regulating neuronal excitability, SK2 channels also modulate synaptic plasticity, a key process underlying learning and memory. SK2 channels are critically involved in the induction of LTP by regulating calcium influx during synaptic activity^{25,51–53}. For instance, SK2 overexpression reduces LTP following high-frequency stimulation²⁵. Consistent with this, mice with blocked SK2 channels required fewer conditioning days to develop significant morphine-context memory (Fig. 2B & C). In contrast, SK2 activation also produced a preference for the morphine-paired context but a nonsignificant decrease in CPP scores (Fig. 3E & F), suggesting that SK2 activation may impair morphine-context memory formation under subthreshold conditions, such as the 3-day paradigm, which is the minimum required to induce MorCPP (Sfig. 2C & D). These results parallel findings in the Morris water maze, where SK2 blockade reduces and overexpression increases the number of training needed to learn^{25,26}. The mechanism underlying SK2's influence on the learning rate of MorCPP likely involves its negative feedback on NMDARs and their role in LTP induction. We previously showed that MorCPP impairs dHPC LTP and increases synaptic NMDARs²⁰. As SK2 channels modulate dendritic NMDARs through a feedback loop that becomes enhanced after CDLSM^{10,23}, SK2 inhibition before conditioning may prevent this feedback, reduce LTP disruption, and thereby facilitate morphine-context learning.

Interestingly, SK2 inhibition produced phase-dependent effects, facilitating acquisition but impairing retrieval of morphine-context memory. This difference may be due to the concurrent presence of systemic morphine during acquisition but not retrieval. Morphine activation of hippocampal μ -opioid receptors (MORs) modulates excitatory and inhibitory inputs and can promote LTP^{54,55}. Although direct MOR-SK2 interactions have not been reported, MOR activity could indirectly influence SK2 through altered NMDA signaling. Thus, SK2 blockade during conditioning may enhance excitability and plasticity in the presence of morphine, promoting

learning, whereas during retrieval (without morphine), the same manipulation disrupts oscillatory coordination necessary for recall. Whether these differential effects arise solely from NMDAR-dependent mechanisms or include MOR-related modulation remains to be determined.

This study has several limitations to consider. First, this study relied on CPP paradigm to assess morphine-context association, and future work should determine whether SK2-dependent modulation extends to other opioid-related paradigms such as CDLSM or intravenous self-administration. Second, electrophysiological experiments were designed to establish an initial link between SK2 inhibition and hippocampal oscillations during morphine-paired context re-exposure. Future experiments involving SK2 activation during both context re-exposure and active choice behavior will be crucial to solidify the link between SK2-dependent network dynamics and the observed behavioral outcomes. Third, the use of single-site electrodes limits layer-specific analysis. As oscillatory powers are known to vary across hippocampal layers, this may also contribute to the observed variability across recordings. Finally, NS309 is not fully SK2-selective and can activate other SK and KCa3.1 channels. Thus, the behavioral effects observed with NS309 may reflect broader SK activation, and future studies using more selective activators will be needed to investigate the role of SK2-specific activation in morphine-context memory.

In conclusion, this study demonstrates that dHPC SK2 channels modulate both the formation and retrieval of morphine-context memories. By regulating NMDAR activity and LTP, SK2 channels likely contribute to the neuronal processes underlying morphine-context association. Our network-level observations complement the behavioral pharmacology findings and point toward a potential mechanism by which SK2 activity may influence drug-context associations. These insights advance our understanding of hippocampal circuits in opioid-associated memory and highlight SK2 channels as potential modulators of context-driven drug behaviors.

Methods

Subjects

All procedures were approved by the Washington University Institutional Animal Care and Use Committee (IACUC) in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. Adult C57BL/6J male mice, aged 10–12 weeks, weighing between 22 and 29 g at the start of every experiment, were from the Moron-Concepcion lab colony or The Jackson Laboratory. Male mice were used in all experiments to maintain consistency with previous studies^{10,20} on which this work builds, allowing comparison of findings. Mice were singly housed post-cannula implants, given access to food pellets and water and libitum, and maintained on a 12/12-hour dark/light cycle (lights on at 7:00 AM). All mice are kept in a sound-attenuated holding facility across the behavior suite 1 week before surgery, post-surgery, and throughout the behavioral assays to minimize stress.

Morphine conditioned place preference (MorCPP) apparatus

Mice were conditioned in two-compartment boxes (63 × 26 × 30.5 cm) in sound-attenuating isolation chambers (MedAssociates). Each conditioning box had a central passageway (8.5 × 26 × 30.5 cm) connecting two distinct compartments for morphine- or saline-paired conditioning (26 × 26 × 30.5 cm). Each compartment had walls with either horizontal or vertical 2.5-cm-wide black and white stripes; these were counterbalanced for side among conditioning boxes. The central passageway walls were matte white. The floors were lined with the same cob bedding as the animals' home cage. The total amount of time spent in each compartment was determined by video-tracking and analyzed with ANY-Maze (Stoelting).

MorCPP protocols

Mice were handled for 7 consecutive days, 5 min per day, during which they were gently restrained and given a subcutaneous saline (s.c.) injection as habituation. Before conditioning, baseline preference for either compartment was determined in a 30-minute pre-test, during which mice were allowed to explore the full apparatus freely. Mice that showed a strong preference for any of the compartments during pre-test (more than 60% of total time spent) were excluded. Morphine vs. saline conditioning and morphine-paired compartment (context) were randomized, unbiased to pre-test preference. To determine the number of conditioning trials required to produce MorCPP, different cohorts of mice underwent 2–4 conditioning days (4–8 sessions total, two per day, spaced 4 h apart). The morning sessions were conducted between 900 and 1245 h, and the afternoon sessions were conducted between 1300 and 1645 h. Morphine-group mice were injected with saline (10 ml/kg; s.c.) in the morning and confined to one compartment for 45 min, and four hours later, injected with 15 mg/kg (10 ml/kg; s.c.) morphine and confined to the other compartment for 45 min. Saline-group mice received saline (10 ml/kg; s.c.) injections during all sessions. This schedule (morning saline, afternoon morphine) was used to avoid potential carry-over or withdrawal effects that could confound conditioning if the order were counterbalanced within the same day. One day after the last conditioning, mice underwent a 30-minute post-test in the morning to assess compartment preference, freely exploring the full apparatus without injections. While investigators could not be blinded to conditioning treatment due to morphine-induced hyperlocomotor activity, behavioral testing was automated using the ANY-Maze video tracking system (Stoelting) with an overhead camera to ensure unbiased data collection and analysis.

Quantitative polymerase chain reaction

Immediately after morCPP post-test, mice were cervically dislocated and decapitated. Bilateral hippocampi were dissected and frozen at –80 °C. RNA was homogenized on QIAshredder spin columns and isolated using the Qiagen RNeasy Mini kit (Qiagen). RNA quality (260/280 ratio > 1.8) and quantity were confirmed using the NanoDrop 2000 (ThermoFisher Scientific). RNA was reverse transcribed into cDNA using the Quanta Bio qScript cDNA Supermix kit (Quantabio) and quantified using Applied Biosystems PowerUp SYBR Green

Master Mix in a QuantStudio 6 Flex Real-Time PCR System (ThermoFisher Scientific). All samples were run in triplicate using the following forward/reverse primers, respectively: SK2, TCT GAT TGC CAG AGT CAT GCT ATT ACA T/CAG TCC ATG CGG CAA TTA TCC A; actin, AGA GGG AAA TCG TGC GTG AC/CAA TAG TGA TGA CCT GGC CGT. Each reaction was programmed to (1) 50 °C (2 min), (2) 95 °C (10 min), (3) 95 °C (15 s), (4) 57 °C (1 min); repeating steps 3 and 4 for 40 cycles; followed by melt curve analysis to confirm specificity of primers. Data were analyzed by $\Delta\Delta C(t)$, normalizing SK2 to actin and comparing morphine vs. saline groups.

Western blotting

Immediately following morCPP post-test, mice were cervically dislocated and rapidly decapitated. Bilateral hippocampi were dissected and frozen at -80 °C. To extract a crude synaptosomal fraction, samples were homogenized in 1 ml Krebs-Ringer Buffer on ice with proteinase and phosphatase inhibitors (125 mM NaCl, 1.2 mM KCl, 1.2 mM MgSO₄, 1.2 mM CaCl₂, 22 mM NaHCO₃, 1 mM NaH₂PO₄, 10 mM glucose, 0.32 M sucrose). Homogenized tissue was spun at 1000 *x g*, 4 °C, for 10 min. Pellet was discarded and supernatant spun again at 16,000 *x g*, 4 °C, for 15 min and then solubilized in 100 μ l Krebs-Ringer Buffer. Protein concentration was determined using the Pierce Bicinchoninic Acid Protein Assay Kit (ThermoFisher Scientific). 10 mg of synaptosomal fraction or total homogenate was loaded onto separate 10% SDS-polyacrylamide gels (125 V, 90 min) and transferred overnight (30 V, 4 °C) to PVDF membranes. Blots were dried, reactivated with methanol, and total protein was measured using the Revert 700 Total Protein Stain (LI-COR). To enable simultaneous probing for SK2 (~64 kD) and actin (~42 kD), blots were segmented at the 50 kD marker (guided by the protein ladder), avoiding the need for stripping and reprobing. After blocking for 1 h with 5% non-fat dry milk in Tris-buffered saline, membranes were incubated with antibodies to SK2 (1:500, rabbit, Alomone) or actin (1:50,000, mouse, Millipore-Sigma) in 5% milk in TBS, overnight at 4 °C on a rocker. Membranes were then washed and probed with WesternSure HRP-Conjugated anti-Rabbit HRP Secondary Antibody and IRDye 800CW anti-Mouse Secondary Antibody, 1:5,000 in Odyssey Blocking Buffer (LI-COR). HRP secondary was detected using WesternSure PREMIUM Chemiluminescent Substrate and both secondaries were imaged on the Odyssey FC (LI-COR). Intensity of signal for SK2 and actin was measured using Image Studio software. SK2 band intensities were normalized to actin and expressed as a ratio of saline controls.

Stereotaxic surgery

Mice were anesthetized with isoflurane (2.5% induction, 1.5-2% maintenance) and secured in a stereotaxic frame (Stoelting). For cannula implantation, bilateral guide cannulae (C232G-3.0/SPC) were positioned above the hippocampus (-1.7 mm AP, \pm 1.5 mm ML, -0.8 mm DV; injector extended 1.0 mm beyond guide). For electrode and combined cannula-electrode implants, a custom assembly (F12792, P1 Technologies) was lowered into dHPC (-1.7 mm AP, -1.5 mm ML, -1.8 mm DV). Additional hole was drilled to secure the ground screw. The guide cannula track was protected with a dummy cannula (C232DC-SPC/8IC315DCMNSP, P1 Technologies) and covered with an aluminum dust cap (303DC/1A, P1 Technologies) while the electrodes were protected with an electrode dust cap (8K00000303DC, P1 Technologies). Implants were secured and affixed to the skull with Metabond dental cement using Radiopaque L-powder, B Quick Base, and C Universal TBB Catalyst (Parkell). Mice were allowed to recover for 7 days prior the start of handling.

Pharmacological manipulation of SK channels

To investigate the role of SK2 channels, both pharmacological inhibition and activation approaches were employed. For SK2 inhibition during the post-test phase, morphine-conditioned mice received bilateral infusions of Lei-Dab⁷ (1 to 20 ng/ μ l, 250 nl per side, Sigma), or vehicle (sterile saline) into dHPC. The infusions were delivered via tailored injectors (C232I/SPC, P1 Technologies) at a rate of 50 nl/min over 5 min using an infusion pump (Harvard Apparatus). Injectors were left in place for another 5 min before mice were placed into the CPP apparatus for a 30-minute session. For conditioning-phase inhibition, Lei-Dab⁷ (10 ng/ μ l) or vehicle was infused bilaterally into the dHPC immediately before the afternoon sessions. Mice were then immediately injected with either morphine or saline (s.c.) and placed in their assigned conditioning compartment for 45 min. To activate the SK2 channels in the dHPC, NS309 (10–150 μ M, 250 nl per side, Sigma), or vehicle (0.7% Dimethyl sulfoxide; DMSO in saline) was infused bilaterally into dHPC immediately before the post-test or the conditioning sessions. NS309 was dissolved in 0.7% DMSO, with lower concentrations prepared by diluting the 150 μ M stock in 0.7% DMSO to maintain a constant vehicle concentration.

Local field potential (LFP) recordings with subcutaneous morphine

Mice were gently restrained, and a cable connected to a commutator was gently screwed onto the electrode. Mice were injected with subcutaneous saline (10 ml/kg) or morphine (15 mg/kg; 10 ml/kg) and dHPC LFP recordings were collected during 30 min of home cage exploration. LFP were collected while mice freely engaged in spontaneous home cage behaviors. The experiment was repeated with reversed treatments 24 h later: mice that initially received saline were administered morphine, and those that initially received morphine were administered saline.

LFP recordings with intra-dHPC Lei-Dab⁷

Mice were gently restrained, and a cable connected to a commutator was gently screwed onto the electrode. Baseline LFPs were recorded for 15 min before saline (vehicle, 250 nl) or Lei-Dab⁷ (10 ng/ μ l, 250 nl, Sigma) infusion with tailored injectors (8IC315IMNSPC, P1 Technologies) through the implanted cannula as previously described. Mice were then reconnected to the commutator and allowed to explore the home cage freely for 30 min while LFPs were recorded. LFP recordings were obtained continuously, with mice allowed to engage in

spontaneous behaviors without restriction to active exploration. 24 h later, to investigate the effect of Lei-Dab⁷ dose on LFP recordings, all mice were infused with Lei-Dab⁷ (1 ng/μl, 250 nl) into the dHPC.

LFP recordings with morphine-context re-exposure

LFP recordings were conducted using the same MorCPP apparatus with two distinct contexts. Mice were initially habituated to both contexts for 30 min to confirm the absence of any inherent contextual bias. During the pre-conditioning recording phase, mice were connected to a commutator to enable LFP recording and were confined to the horizontal compartment in the morning and the vertical compartment in the afternoon. This was followed by a 4-day conditioning period (saline in the morning and morphine in the afternoon; context counterbalanced). Prior to post-conditioning recording, Lei-Dab⁷ (10 ng/μl, 250 nl; Sigma) or saline (vehicle, 250 nl) was infused over 5 min using a pump, as described previously. Immediately after infusion, the animal was connected to a commutator and placed in the horizontal compartment for morning recordings or the vertical compartment for afternoon recordings. As mice were confined to and allowed to freely explore a single compartment at a time during both pre- and post-conditioning recordings, they were not able to choose between contexts, and CPP scores were not calculated for this experiment.

LFP recording and analysis

LFPs were recorded continuously from the dHPC using an RZ5P processor (Tucker-Davis Technologies), with wide-band neuronal signals (0.1–300 Hz) acquired via implanted electrodes, amplified (×2) using a PZ5 amplifier (Tucker-Davis Technologies), digitized at 1017 Hz for offline analysis. Recordings were obtained during spontaneous behavior, and analyses were not restricted to periods of locomotion or active exploration. Data processing was performed using custom scripts in MATLAB (MATLAB R2019b, MathWorks). Raw signals were initially band-pass filtered (1–300 Hz), followed by a second-order Butterworth notch filter to remove 60 Hz noise. Power spectral density (PSD) was computed using the Welch periodogram method (*pwelch* function, Signal Processing Toolbox) with 4-s Hanning windows and 50% overlap⁵⁶. The total power within the theta (4–13 Hz), low gamma (30–60 Hz), and high gamma (60–100 Hz) frequency bands was extracted for subsequent statistical comparison. For phase-amplitude coupling (PAC) analysis, instantaneous theta phase and gamma amplitude time series were derived using the Hilbert transform (*hilbert* function, Signal Processing Toolbox). PAC strength was quantified using the modulation index (MI) described in^{34,35}. theta phases were divided into 18 bins of 20° (0–360°), and the mean gamma amplitude in each bin was calculated. These values were normalized by the sum across bins, resulting in a probability distribution of gamma amplitude across theta phase (see S Figs. 3 and 5). To measure how much the phase-amplitude distribution deviates from the uniform distribution, the Kullback-Leibler (KL) distance was used^{35,57}. The MI was calculated by dividing the KL distance by a constant factor (logarithm of the number of bins), resulting in values between 0 and 1. A MI value of 0 denotes that the mean amplitude is the same for all phase bins and that there is an absence of phase-amplitude coupling (the phase-amplitude distribution is equal to a uniform distribution). To compare across groups, MI values were normalized within each mouse by dividing each group value by the average of its paired conditions, allowing statistical comparisons with t-tests or ANOVA³⁵.

Data analysis

All experiments and treatments were conducted in at least two separate cohorts of mice to control for potential day- or condition-specific variability. Mice were randomly assigned to treatment groups and CPP boxes before testing. Statistical analyses were performed using GraphPad Prism (v10.4), with data presented as mean ± S.E.M. or mean with before-after pairing and statistical significance defined as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$. Behavioral CPP results were analyzed using one-way, two-way, or three-way RM ANOVAs, followed by post-hoc tests (Tukey, Bonferroni, or Šidák) for group comparisons. If there is no interaction but a main effect of a factor, post-hoc tests were applied to the relevant factor⁵⁸. CPP preference scores (post-pre) were additionally tested against a hypothetical value of 0 (indicating no preference) using one-sample t-test or Wilcoxon signed-rank tests. Molecular data (qPCR and Western blot) were analyzed using two-tailed unpaired t-tests. LFP and PAC results were analyzed using two-tailed paired t-tests or repeated-measures ANOVAs, followed by Šidák post hoc tests, with non-parametric equivalents applied when assumptions were violated. Correlations were evaluated using Pearson or Spearman coefficients. Sample sizes were determined using WebPower based on a significance level of 0.05, power of 0.8, and an expected medium effect size (Cohen's $d = 0.5–0.7$)⁵⁹, consistent with prior MorCPP studies^{20,60}. Detailed statistics, including n numbers, are reported in figure legends, with full analysis outputs provided in supplementary materials.

Data availability

Authors can confirm that all relevant data are included in the paper and its supplementary information files.

Received: 10 July 2025; Accepted: 15 October 2025

Published online: 20 November 2025

References

1. Lipari, R. N. Key Substance Use and Mental Health Indicators in the United States: Results from the 2018 National Survey on Drug Use and Health. 82. (2018).
2. Wilson, N. Drug and Opioid-Involved overdose Deaths — United States, 2017–2018. *MMWR Morb Mortal. Wkly. Rep.* **69**. (2020).
3. Blanco, C. & Volkow, N. D. Management of opioid use disorder in the USA: present status and future directions. *Lancet* **393**, 1760–1772 (2019).
4. Dacher, M. & Nugent, F. S. Opiates and plasticity. *Neuropharmacology* **61**, 1088–1096 (2011).

5. Volkow, N. D., Jones, E. B., Einstein, E. B. & Wargo, E. M. Prevention and treatment of opioid misuse and addiction: A review. *JAMA Psychiatry*. **76**, 208 (2019).
6. Childress, A. R. et al. Cue reactivity and cue reactivity interventions in drug dependence. *NIDA Res. Monogr.* **137**, 73–95 (1993).
7. Taubenfeld, S. M., Muravieva, E. V., Garcia-Osta, A. & Alberini, C. M. Disrupting the memory of places induced by drugs of abuse weakens motivational withdrawal in a context-dependent manner. *PNAS* **107**, 12345–12350 (2010).
8. Daghli, M. R. C. et al. Changes in regional cerebral blood flow elicited by craving memories in abstinent Opiate-Dependent subjects. *AJP* **158**, 1680–1686 (2001).
9. See, R. E. Neural substrates of conditioned-cued relapse to drug-seeking behavior. *Pharmacol. Biochem. Behav.* **71**, 517–529 (2002).
10. Fakira, A. K., Portugal, G. S., Carusillo, B., Melyan, Z. & Morón, J. A. Increased small conductance Calcium-Activated potassium type 2 Channel-Mediated negative feedback on N-methyl-D-aspartate receptors impairs synaptic plasticity following Context-Dependent sensitization to morphine. *Biol. Psychiatry*. **75**, 105–114 (2014).
11. Masukawa, M. Y., Correa-Netto, N. F., Silva-Gomes, A. M., Linardi, A. & Santos-Junior, J. G. Anxiety-like behavior in acute and protracted withdrawal after morphine-induced locomotor sensitization in C57BL/6 male mice: the role of context. *Pharmacol. Biochem. Behav.* **194**, 172941 (2020).
12. Trombin, T. F. et al. Environmental novelty modulates the induction and expression of single injection-induced behavioral sensitization to morphine. *Pharmacol. Biochem. Behav.* **173**, 90–95 (2018).
13. Chefer, V. I. & Shippenberg, T. S. Augmentation of morphine-Induced sensitization but reduction in morphine tolerance and reward in Delta-Opioid receptor knockout mice. *Neuropsychopharmacol* **34**, 887–898 (2009).
14. Robinson, T. E. & Berridge, K. C. The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Res. Rev.* **18**, 247–291 (1993).
15. Tzschentke, T. M. REVIEW ON CPP: measuring reward with the conditioned place preference (CPP) paradigm: update of the last decade. *Addict. Biol.* **12**, 227–462 (2007).
16. Kim, H.-S., Jang, C.-G. & Park, W.-K. Inhibition by MK-801 of morphine-induced conditioned place preference and postsynaptic dopamine receptor supersensitivity in mice. *Pharmacol. Biochem. Behav.* **55**, 11–17 (1996).
17. Ribeiro Do Couto, B., Aguilar, M. A., Manzanedo, C., Rodríguez-Arias, M. & Miñarro, J. NMDA glutamate but not dopamine antagonists blocks drug-induced reinstatement of morphine place preference. *Brain Res. Bull.* **64**, 493–503 (2005).
18. Benturquia, N. et al. Specific Blockade of morphine- and cocaine-induced reinforcing effects in conditioned place preference by nitrous oxide in mice. *Neuroscience* **149**, 477–486 (2007).
19. Solecki, W., Turek, A., Kubik, J. & Przewlocki, R. Motivational effects of opiates in conditioned place preference and aversion paradigm—a study in three inbred strains of mice. *Psychopharmacology* **207**, 245 (2009).
20. Portugal, G. S. et al. Hippocampal Long-Term potentiation is disrupted during expression and extinction but is restored after reinstatement of morphine place preference. *J. Neurosci.* **34**, 527–538 (2014).
21. Xia, Y. et al. Hippocampal GluA1-Containing AMPA receptors mediate Context-Dependent sensitization to morphine. *J. Neurosci.* **31**, 16279–16291 (2011).
22. Pedarzani, P. et al. Specific enhancement of SK channel activity selectively potentiates the afterhyperpolarizing current IAHP and modulates the firing properties of hippocampal pyramidal neurons. *J. Biol. Chem.* **280**, 41404–41411 (2005).
23. Ngo-Anh, T. J. et al. SK channels and NMDA receptors form a Ca²⁺-mediated feedback loop in dendritic spines. *Nat. Neurosci.* **8**, 642–649 (2005).
24. McKay, B. M. et al. Increasing SK2 channel activity impairs associative learning. *J. Neurophysiol.* **108**, 863–870 (2012).
25. Hammond, R. S. et al. Small-Conductance Ca²⁺-Activated K⁺ channel type 2 (SK2) modulates hippocampal Learning, Memory, and synaptic plasticity. *J. Neurosci.* **26**, 1844 (2006).
26. Stackman, R. W. et al. Small conductance Ca²⁺-activated K⁺ channels modulate synaptic plasticity and memory encoding. *J. Neurosci.* **22**, 10163–10171 (2002).
27. Murthy, S. R. K. et al. Small-Conductance Ca²⁺-Activated potassium type 2 channels regulate the formation of contextual fear memory. *PLOS ONE*. **10**, e0127264 (2015).
28. Oliveira, M. S. et al. Altered expression and function of small-conductance (SK) Ca²⁺-activated K⁺ channels in pilocarpine-treated epileptic rats. *Brain Res.* **1348**, 187–199 (2010).
29. Qu, L. et al. Altered activity of SK channel underpins morphine withdrawal relevant psychiatric deficiency in infralimbic to accumbens shell pathway. *Front. Psychiatr.* ;**10**. (2019).
30. Vogel, C. & Marcotte, E. M. Insights into the regulation of protein abundance from proteomic and transcriptomic analyses. *Nat. Rev. Genet.* **13**, 227–232 (2012).
31. Ridley, R. M., Timothy, C. J., Maclean, C. J. & Baker, H. F. Conditional learning and memory impairments following neurotoxic lesion of the CA1 field of the hippocampus. *Neuroscience* **67**, 263–275 (1995).
32. Sakimoto, Y., Takeda, K., Okada, K., Hattori, M. & Sakata, S. Transient decline in rats' hippocampal theta power relates to inhibitory stimulus-reward association. *Behav. Brain Res.* **246**, 132–138 (2013).
33. Liu, F., Jiang, H., Zhong, W., Wu, X. & Luo, J. Changes in ensemble activity of hippocampus CA1 neurons induced by chronic morphine administration in freely behaving mice. *Neuroscience* **171**, 747–759 (2010).
34. Tort, A. B. L. et al. Dynamic cross-frequency couplings of local field potential oscillations in rat striatum and hippocampus during performance of a T-maze task. *Proc. Natl. Acad. Sci.* **105**:20517–20522. (2008).
35. Tort, A. B. L., Komorowski, R. W., Manns, J. R., Kopell, N. J. & Eichenbaum, H. Theta–gamma coupling increases during the learning of item–context associations. *Proc. Natl. Acad. Sci.* **106**:20942–20947. (2009).
36. Hentschke, H., Perkins, M. G., Pearce, R. A. & Banks, M. I. Muscarinic Blockade weakens interaction of gamma with theta rhythms in mouse hippocampus. *Eur. J. Neurosci.* **26**, 1642–1656 (2007).
37. Buzsáki, G. et al. Hippocampal network patterns of activity in the mouse. *Neuroscience* **116**, 201–211 (2003).
38. Dragoi, G. & Buzsáki, G. Temporal encoding of place sequences by hippocampal cell assemblies. *Neuron* **50**, 145–157 (2006).
39. Lisman, J. E. & Idiart, M. A. P. Storage of 7 ± 2 Short-Term memories in oscillatory subcycles. *Science* **267**, 1512–1515 (1995).
40. O'Keefe, J. & Recce, M. L. Phase relationship between hippocampal place units and the EEG theta rhythm. *Hippocampus* **3**, 317–330 (1993).
41. Buzsáki, G. Theta oscillations in the hippocampus. *Neuron* **33**, 325–340 (2002).
42. Howard, M. W. et al. Gamma oscillations correlate with working memory load in humans. *Cereb. Cortex.* **13**, 1369–1374 (2003).
43. Zhang, J.-J. & Kong, Q. Locomotor activity: A distinctive index in morphine self-administration in rats. *PLOS ONE*. **12**, e0174272 (2017).
44. Mizoguchi, H. et al. Lack of a rewarding effect and a locomotor-enhancing effect of the selective μ-opioid receptor agonist amidino-TAPA. *Psychopharmacology* **212**, 215–225 (2010).
45. McFarland, W. L., Teitelbaum, H. & Hedges, E. K. Relationship between hippocampal theta activity and running speed in the rat. *J. Comp. Physiol. Psychol.* **88**, 324–328 (1975).
46. Vanderwolf, C. H. Hippocampal electrical activity and voluntary movement in the rat. *Electroencephalogr. Clin. Neurophysiol.* **26**, 407–418 (1969).
47. Bouwman, B. M. et al. The relationship between hippocampal EEG theta activity and locomotor behaviour in freely moving rats: effects of Vigabatrin. *Brain Res. Bull.* **64**, 505–509 (2005).
48. Lin, M. T. et al. Coupled Activity-Dependent trafficking of synaptic SK2 channels and AMPA receptors. *J. Neurosci.* **30**, 11726–11734 (2010).

49. Stocker, M., Krause, M. & Pedarzani, P. An apamin-sensitive Ca²⁺-activated K⁺ current in hippocampal pyramidal neurons. *Proc. Natl. Acad. Sci.* **96**, 4662–4667 (1999).
50. Sun, J., Liu, Y., Baudry, M. & Bi, X. SK2 channel regulation of neuronal excitability, synaptic transmission, and brain rhythmic activity in health and diseases. *Biochim. Biophys. Acta Mol. Cell. Res.* **1867**, 118834 (2020).
51. Lin, M. T., Luján, R., Watanabe, M., Adelman, J. P. & Maylie, J. SK2 channel plasticity contributes to LTP at Schaffer collateral-CA1 synapses. *Nat. Neurosci.* **11**, 170–177 (2008).
52. Malenka, R. C. & Nicoll Long-Term Potentiation—A decade of progress? *Science* **285**, 1870–1874 (1999).
53. Nicoll, R. A. A brief history of Long-Term potentiation. *Neuron* **93**, 281–290 (2017).
54. Kim, H. R., Dey, S., Sekerkova, G. & Martina, M. μ -Opioid receptor modulation of the Glutamatergic/GABAergic midbrain inputs to the mouse dorsal hippocampus. *J. Neurosci.* **44**, e0653242024 (2024).
55. Capogna, M., Gähwiler, B. H. & Thompson, S. M. Mechanism of mu-opioid receptor-mediated presynaptic Inhibition in the rat hippocampus in vitro. *J. Physiol.* **470**, 539–558 (1993).
56. Mesgar, S., Eskandari, K., Karimian-Sani-Varjovi, H., Salemi-Mokri-Boukani, P. & Haghparast, A. The dopaminergic system modulates the electrophysiological activity of the Suprachiasmatic nucleus dependent on the circadian cycle. *Neurochem Res.* **48**, 3420–3429 (2023).
57. Santner, T. J. & Duffy, D. E. *The Statistical Analysis of Discrete Data* (Springer Science & Business Media, 2012).
58. Wei, J., Carroll, R. J., Harden, K. K. & Wu, G. Comparisons of treatment means when factors do not interact in two-factorial studies. *Amino Acids.* **42**, 2031–2035 (2012).
59. Zhang, Z. & Yuan, K-H. *Practical Statistical Power Analysis Using Webpower and R* (ISDSA, 2018).
60. Sakae, D. Y. & Martin, S. J. Formation of a morphine-conditioned place preference does not change the size of evoked potentials in the ventral hippocampus–nucleus accumbens projection. *Sci. Rep.* **9**, 5206 (2019).

Acknowledgements

We would like to thank all members of the Moron-Concepcion's lab for their help and support throughout the completion of the current study. This manuscript is the result of funding in whole or in part by the National Institutes of Health (NIH) grant R01DA058613 (J.A.M.), R01DA042499 (J.A.M.), R01DA054900 (J.A.M.) and McDonnell Small Grant award (K.M.I.). It is subject to the NIH Public Access Policy. Through acceptance of this federal funding, NIH has been given a right to make this manuscript publicly available in PubMed Central upon the Official Date of Publication, as defined by NIH. The schematic in Supplementary Figure 1A was created with the help of images from BioRender.com (<https://BioRender.com/zgeyv0>). All other illustrations and schematics were created by author, Khairunisa Mohamad Ibrahim, using Affinity Designer 2 (Version 2.5). The study is reported in accordance with ARRIVE guidelines.

Author contributions

Conceptualization: K.M.I., D.J.B., N.M. and J.A.M. Methodology: K.M.I., D.J.B., S.W., N.M. and J.A.M. Formal analysis: K.M.I. and D.J.B. Investigation: K.M.I., D.J.B., A.Z., O.I., J.D.M. and W.W.P. Writing – Original Draft: K.M.I. Writing – Revisions: K.M.I. Writing – Reviewing & Editing: K.M.I., D.J.B., N.M. and J.A.M. Illustrations & Figures: K.M.I. Funding, Resources & Supervision: K.M.I. and J.A.M.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1038/s41598-025-24819-6>.

Correspondence and requests for materials should be addressed to J.A.M.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

© The Author(s) 2025