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In vivo administration of a subtype selective activator of small conductance Ca2+-activated K+ channels influences hippocampal-dependent spatial memory

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Small conductance calcium activated potassium (SK) channels constrain learning and memory in several tasks. Past research found mice overexpressing the SK2 channel, one of three SK subunits expressed in the CNS (SK1, SK2, and SK3), or receiving systemic treatment with broad range positive SK channel modulators express deficits in hippocampal dependent memory tasks. The effects of the more selective SK2/SK3 activator cyclohexyl-[2-(3,5-dimethyl-pyrazol-1-yl)-6-methyl-pyrimidin-4-yl]-amine (CyPPA) were examined in male C57BL/6J mice in a behavioral task battery. Systemic CyPPA produced no significant deficits in the hippocampal dependent Morris water maze task with mice exhibiting equivalent platform search accuracy during a probe test. However, CyPPA disrupted the typical directional responding observed during the water maze probe test. To test the effects of CyPPA on conditioned fear memory, 4 separate cohorts were administered systemic CyPPA or vehicle before pre-exposure to the conditioning chamber, before tone-shock pairing, or before the tone/context tests. Mice administered CyPPA prior to tone/context testing exhibited reduced contextual freezing relative to vehicle-treated control mice and all 3 CyPPA cohorts produced higher percent freezing than the control during the tone test. These data support past evidence of SK channel's role in non-aversive memory and reveal a unique role in aversive memory.

In vivo administration of a subtype selective activator of small conductance Ca^{2+}-activated K^+ channels influences hippocampal-dependent spatial memory

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Introduction

Activation of small conductance calcium activated potassium (SK) channels constrain learning and memory in several tasks. Past research found mice overexpressing the SK2 channel, one of three SK subunits expressed in the CNS (SK1, SK2, and SK3), or receiving systemic treatment with 1-EBIO, a broad range positive SK channel modulator, express deficits in hippocampal dependent memory tasks (Hammond et al., 2006, *J. Neurosci.*; Vick et al., 2010, *Neuropharm*). The effects of the more selective SK2/SK3 activator cyclohexyl-[2-(3,5-dimethyl-pyrazol-1-yl)-6-methyl-pyrimidin-4-yl]-amine (CyPPA) were examined in male C57BL/6J mice in a behavioral task battery designed to test hippocampal and amygdala dependent memory.

Methods

Hippocampal-dependent memory: The Morris water maze task was utilized to test the effects of CyPPA on hippocampal-dependent memory. Mice were habituated to the water maze over a 2 day period with each day consisting of 4 trials. During each day of training, mice received either systemic CyPPA (15mg/kg, i.p.) or vehicle 30 minutes prior to entering the arena and underwent four trials starting from non-sequential locations. Experiment 1 consisted of 9 days of training followed by a probe test (removing the platform) on the 1st, 3rd, 5th, and 7th days of training to test the temporal effects of CyPPA on learning and memory throughout the process. After the final day of training, mice underwent a pool-shift probe to explore CyPPA's effects on directional and place preference. Experiment 2 was designed to investigate CyPPA's involvement in memory formation, and to see if CyPPA was involved in memory retrieval. This experiment also consisted of 9 days of training, however, after the 4th day of training, mice receiving CyPPA began receiving vehicle and mice receiving vehicle began receiving CyPPA.

Amygdala-dependent memory: A Pavlovian conditioning paradigm was utilized to test the effects of CyPPA on conditioned fear memory. Mice were separated into 4 separate cohorts that were administered systemic CyPPA or vehicle before pre-exposure to the conditioning chamber, before tone-shock pairing, or before the tone and context tests. On the first day, mice were allowed to freely explore the conditioning chamber for 5 minutes. During pairing, mice were exposed to 3 CS-US pairings each consisting of a 30 second tone (90 dB, 5000 Hz, CS) that co-terminated with a 1-s, 0.5mA footshock (US), and separated by a 120-s inter-stimulus interval (ISI). 24 hours after conditioning, mice were returned to same chamber for 5 minutes, without the presentation of a tone or footshock, to test contextual memory. The context test was followed by a tone test where mice were placed into a novel, modified chamber containing a white Plexiglas floor, a black Plexiglas triangular insert to alter lighting, and were cleaned with 1% LiquiNox to alter odor cues, where the mice were exposed to the same CS twice for 30 seconds separated by a 120-s ISI.

Systemic CyPPA during Hippocampal Dependent Task

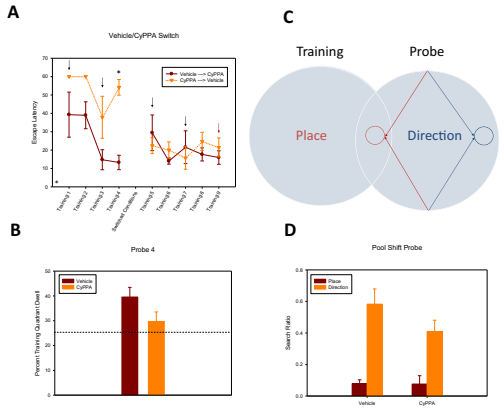

Figure 1. **Systemic CyPPA did not disrupt hippocampal dependent memory. (A)** Mice initially receiving systemic CyPPA showed deficits when compared to mice receiving vehicle during training ($F(1, 3) = 60.15$, $p < .01$), with the largest difference occurring during the 4th day, ($t(6) = 7.052$, $p < .001$). This deficit was reversed and no significant difference between the cohorts existed past the 5th day of training ($F(1, 3) = .04$, *n.s.*), when mice previously receiving CyPPA were now administered vehicle and mice receiving vehicle were now administered CyPPA. **(B)** A two-factor repeated measures ANOVA found a significant effect for CyPPA during the probe test ($F(1, 4) = 9.35$, $p < .05$). A significant difference was found between the CyPPA and vehicle groups during the probe test, however, no significant difference was found during probe 1 ($t(10) = .60$, *n.s.*), probe 2 ($t(10) = -.06$, *n.s.*), or probe 3 ($t(10) = -2.09$, *n.s.*), but there was a significant difference during probe 4 ($t(10) = 2.78$, $p < .05$); dotted line indicates chance performance. **(C)** Mice are trained with the pool located in the western position and platform located in the eastern quadrant. Pool is shifted to the east and the platform is removed during probe. Extra-maze cues remain the same and mice are scored by the amount of time spent in either the East or West quadrant, which is analyzed to determine a preference for direction or place, respectively. **(D)** Mice preferred direction over place for both the vehicle ($t(8) = 5.05$, $p < .001$) and CyPPA ($t(8) = 3.76$, $p < .01$) cohorts. The two groups did not significantly differ on their preference for direction ($t(8) = 1.44$, *n.s.*) or place ($t(8) = .04$, *n.s.*). During the pool-shift probe, mice receiving CyPPA did not show a significant difference in preference when compared to mice receiving vehicle.

Systemic CyPPA during Amygdala Dependent Task

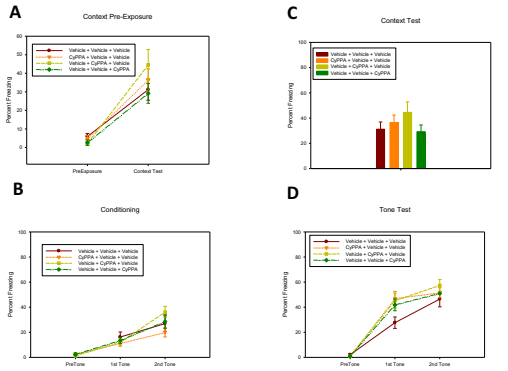

Figure 2. **Systemic CyPPA did not impair any stage of aversive memory formation (A)** A two-factor repeated measures ANOVA found no significant difference between cohorts ($F(1, 13) = .10$, *n.s*) between pre-exposure and the context test. **(B)** Regression analysis found no significant difference in the percent freezing between any of the 4 cohorts during conditioning ($F(1, 14) = .01$, *n.s.*). **(C)** A one-way ANOVA found no significant difference during the contextual memory task ($F(58, 3) = 1.163$, *n.s.*). **(D)** Regression analysis found no significant difference in the percent freezing between any of the cohorts during tone test ($F(1, 13) = 2.13$, *n.s.*). The increase in freezing for all mice injected with systemic CyPPA during the first tone was also insignificant ($F(3, 57) = 2.117$, *n.s.*), however, during the first tone, mice under the influence of CyPPA during pre-exposure had a significantly higher percent freezing compared to control, $t(28) = -2.397$, $p < .05$.

Conclusion

- As previously discovered, SK channel activation impairs hippocampal dependent memory, however, CyPPA, an SK2 and SK3 subtype-specific activator, did not induce as extensive of an impairment to spatial memory as recorded in mice overexpressing SK2 (Hammond et al., 2006) or mice receiving a broad range positive SK channel modulator (Vick et al., 2010), nor the expected deficit to fear memory found in mice overexpressing SK2 (Stackman et al., 2008).
- Mice receiving systemic CyPPA performed at chance during the final probe test while mice receiving vehicle showed a preference for the correct quadrant, indicating that CyPPA impaired spatial memory retrieval.
- The deficits exhibited by mice receiving systemic CyPPA on a hippocampal-dependent memory task were quickly recovered when mice were switched to vehicle, signifying that CyPPA influenced memory encoding.
- Mice who had previously learned the task did not exhibit any deficits when switched to CyPPA, signifying that CyPPA did not influence memory retrieval.
- The current study found no significant effect for systemic CyPPA on the amygdala-dependent task, which is consistent with Vick et al. (2010) and Atchley et al (2012), where systemic treatment with SK channel activators did not impair the encoding of fear memory, indicating limitations to systemic treatment with SK activators.

Future Direction

- Test the role of CyPPA and other SK channel modulators under a more diverse battery of behavioral tasks.
- Infuse SK channel modulators directly into the hippocampus, amygdala, and perirhinal cortex before encoding or retrieval of memory associated with a variety of tasks.
- Record cells in the hippocampus, amygdala, and perirhinal cortex during different stages of memory and learning across a variety of tasks.
- Future research must assess the role SK channels play in different types of memory and determine how the influence of SK channels varies across an eclectic variety of memory-dependent behavioral tasks.