Kappa-opioid receptor stimulation in the nucleus accumbens shell: Differential effects across the rostro-caudal axis on dopamine transmission and affective behavior

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ABSTRACT

Neural activity in the nucleus accumbens (NAc) shell has long been implicated in the regulation of negative and positive affect. Classically, the dynorphin/kappa opioid receptor (KOR) system in the NAc was believed to promote dysphoric behaviors, while dopamine release was viewed as interacting with reinforcing stimuli, and KOR-activation was known to inhibit dopamine release. Recently, however, both the KOR and dopamine systems have been shown to have differential effects across the rostro-caudal axis of the NAc shell on hedonic responses. Whether or not this is due to interactions between KORs and dopamine, and if it extends to other affective behaviors, remains to be determined. In this study, we examined in rats the relationship between the KOR and dopamine systems in both the rostral and caudal NAc shell using *ex vivo* fast scan cyclic voltammetry and the impact of KOR activation on affective behaviors using various assays. We found that activation of KORs in the caudal NAc shell resulted in greater dopamine inhibition, and stimulated novelty-induced rearing behavior and anxiety-like and avoidance behaviors while reducing locomotor activity. In contrast, activation of KORs in the rostral NAc shell resulted in less dopamine inhibition and actually reduced anxiety-like and avoidance behaviors. These results demonstrate that there is heterogeneity across the rostro-caudal axis of the NAc shell in the effects of KOR stimulation on affective behavior and they suggest that this might be due to differences in KOR control over dopamine inhibition.

INTRODUCTION

The nucleus accumbens (NAc) processes responses to both aversive and rewarding stimuli, but the mechanisms through which this occurs remain to be determined. A heterogenous area, the NAc receives inputs of the monoamine dopamine from the ventral tegmental area and contains cell bodies and axon terminals of neurons producing the neuropeptide dynorphin (Van Bockstaele et al., 1995), which acts primarily at the kappa opioid receptor (KOR) (Chavkin et al., 1982). Mesolimbic dopamine, dynorphin/KORs, and their interplay are involved in affective states (Chartoff et al., 2016; Karkhanis, Rose, et al., 2016). While dopamine release in the NAc is facilitated by both reinforcing and aversive stimuli (Margolis & Karkhanis, 2019), KOR activation promotes both preference and aversion (Castro & Bruchas, 2019). Thus, both dopamine and the KORs may be involved in the ability of the NAc to process aversive and rewarding stimuli, but it remains to be determined how this occurs.

Classically, the dynorphin/KOR system was believed to promote dysphoric behaviors. Activation of KORs in the NAc potentiated conditioned place aversion (Bals-Kubik et al., 1993; Chefer et al., 2013; Ehrich et al., 2015) and inhibition suppressed depressive- and anxiety-like behaviors (Land et al., 2008). More recently, however, studies have shown that KOR activation can have opposing effects (Al-Hasani et al., 2015; Castro & Berridge, 2014). In the middle NAc shell of mice, optical stimulation of dynorphin-releasing medium spiny neurons in the dorsal subregion was found to augment conditioned and real-time place preference, while stimulation in the ventral subregion promoted aversion (Al-Hasani et al., 2015). In rats, KOR activation in rostral half of the NAc shell enhanced hedonic orofacial reactions to sucrose while activation in caudal half suppressed them (Castro & Berridge, 2014). These studies suggest that KOR activation may make distinct contributions to affective behavior via different anatomical subregions of the NAc shell, but they highlight the need to understand the mechanisms driving such opposing behaviors.

Within the NAc, KORs regulate the release of various neurotransmitters, including dopamine. Acting as inhibitory G-protein coupled receptors (Cox, 2013; Wu et al., 2012), KORs in the NAc are located on dopamine terminals (Svingos et al., 2001) and their activation inhibits dopamine release (Di Chiara & Imperato, 1988a; Karkhanis, Huggins, et al., 2016; Karkhanis, Rose, et al., 2016; Rose et al., 2016; Spanagel et al., 1992). While dopamine release in the NAc has classically been viewed as being responsive to reinforcing stimuli (Di Chiara & Imperato, 1988b; Karkhanis et al., 2014; Yim et al., 2000), it has more recently been found also to be responsive to aversive stimuli (Margolis & Karkhanis, 2019; Mikhailova et al., 2019; Moriya et al., 2018). Furthermore, while dopamine release in the rostral NAc shell was shown to inhibit taste aversion, dopamine in caudal shell instead enhanced it (Hurley et al., 2017). Thus, like KORs,

dopamine in the NAc is involved in encoding both aversive and rewarding responses, and this may occur via different subregions of the NAc shell.

In this study, we sought to pinpoint a mechanism that may be involved in the opposing behavioral effects of KOR activation in the NAc shell. Our hypothesis was that KOR activation in the rostral compared to caudal subregion of the NAc shell results in different or even opposite effects on both dopamine transmission and affective behavior.

MATERIALS AND METHODS

Subjects

Adult, male and female Long-Evans rats (*N* = 43; 38 males and 5 females; 8 weeks at the start of testing, Charles River Laboratories International, Inc., Malvern, PA, USA and Kingston, NY, USA) were pair housed (for neurobiological studies) or individually housed (for behavioral studies) in an AAALAC-accredited facility on a 12-hour reversed light/dark cycle (lights off at 0300 h for neurobiological studies or 0900 h for behavioral studies). They were given at least one week to acclimate to the facility and were handled daily prior to the start of experiments. Rats received *ad libitum* chow (Laboratory Rodent Diet 5001, Lab Diet, St. Louis, MO, USA) and water throughout the study. Experiments were approved by the Institutional Animal Care and Use Committees of SUNY Binghamton University and Drexel University College of Medicine and followed the NIH Guide for the Care and Use of Laboratory Animals.

Behavioral experimental protocol

Experiment 1

To determine the effects of KOR stimulation on dopamine dynamics in the rostral compared to caudal NAc shell, male and female rats (*n* = 5/group) were sacrificed and *ex vivo* fast scan cyclic voltammetry (FSCV) was used to measure dopamine release under baseline conditions and in the presence of the selective KOR agonist, U50,488 (Von Voigtlander & Lewis, 1982; Vonvoigtlander et al., 1983).

Experiment 2

To determine the effects of KOR stimulation on novelty-induced locomotor activity, male rats (N = 33) were injected in the rostral or caudal NAc shell between-subject with U50,488 or saline vehicle (n = 6 – 12/group) and tested in a novel activity chamber.

Experiment 3

To determine the effects of KOR stimulation on anxiety-like or avoidance behavior, some rats from Experiment 2 (N = 17) were, one week later, injected in the rostral or caudal NAc shell within-subject with U50,488 or saline vehicle (n = 6 – 11/group) and tested in a light-dark box.

Experiment 4

To determine the effects of KOR stimulation on another measure of anxiety-like or avoidance behavior as well as locomotor activity, rats (N = 20) were injected in the rostral or caudal NAc shell within-subject with U50,488 or saline vehicle (n = 7 – 13/group) and tested in an open field. While 11 of these rats were tested one week after the novel activity chamber (Experiment 2), 9 were tested one week after the light-dark box (Experiment 3).

Ex vivo fast scan cyclic voltammetry

Ex vivo FSCV was used to characterize the functionality of KORs in the rostral and caudal NAc shell. Briefly, rats were sacrificed 6 hours into the dark cycle and their brains were rapidly removed and prepared as previously described (John & Jones, 2007). A vibrating tissue slicer was used to prepare 400 µm thick coronal brain sections containing NAc. These slices were immersed in oxygenated artificial cerebrospinal fluid (aCSF; 32°C) containing: 126 mM NaCl, 2.5 mM KCl, 1.2 mM NaH_2PO_4, 2.4 mM $CaCl_2$, 1.2 mM $MgCl_2$, 25 mM $NaHCO_3$, 11 mM glucose, 0.4 mM L-ascorbic acid and the pH was adjusted to 7.4. Endogenous dopamine release was evoked by single electrical pulse stimulation (monophasic+, 4 ms, 750 µA) applied to the tissue every 5 minutes and dopamine was detected using a triangular scanning waveform (-0.4 to +1.2 and back to -0.4 V, Ag *vs* AgCl) at the rate of 400 V/s. Recording electrodes were calibrated with known concentrations of dopamine (3 µM). After stable dopamine responses were obtained, cumulative concentrations of the KOR agonist, U50,488 (0.01-1.0 µM; Tocris, Minneapolis, MN, USA), were bath applied to rostral and caudal NAc shell slices. All FSCV data were analyzed using a Michaelis-Menten kinetics-based

algorithm (Ferris et al., 2013), using Demon Voltammetry and Analysis software (Yorgason et al., 2011). Recording sites for FSCV are shown in **Figure 1A**.

Microinjections

Rats were cannulated bilaterally in the rostral or caudal NAc shell, using published methods (Pandey et al., 2019). Stainless steel guide shafts (21–gauge, Small Parts) were implanted perpendicularly, aimed at the rostral NAc shell (2.1 mm anterior to bregma, ±0.8 mm lateral to midline, 4.0 mm ventral to the level skull) or caudal NAc shell (1.1 mm anterior to bregma, ±0.8 mm lateral to midline, 4.0 mm ventral to the level skull). To prevent occlusion, 26–gauge stainless steel stylets were left in the guide shafts between injections. During their one week recovery period, rats were handled daily, and their stylets were removed and replaced to acclimate them to the microinjection procedure.

Injections of freshly-prepared drug (8.0 nmol U50,488; Tocris, Minneapolis, MN, USA) or saline vehicle (Baxter International Inc., Deerfield, IL, USA) were given 2 hours into the dark cycle through concentric microinjectors of 26–gauge stainless steel outside and fused-silica tubing inside (74 μm ID, 154 μm OD; Polymicro Technologies, Phoenix, AZ, USA) that protruded 3.0 mm beyond the guide shafts to reach the NAc shell. A syringe pump (Harvard Apparatus, Holliston, MA, USA) delivered 0.3 μl of solution over 30 seconds, and the microinjector remained in place for an additional 30 – 60 seconds to allow for diffusion. Each hemisphere was injected sequentially for each subject. We have previously demonstrated that injection of methylene blue dye at the volume used (0.3 μl) has a radial spread of approximately 0.5 mm (Barson et al., 2015; Barson & Leibowitz, 2015). Behavioral testing for each subject began 15 minutes following the completion of their second injection.

Behavioral testing

Novelty-Induced Locomotor Activity

Novelty-induced locomotor activity was measured in an activity chamber with an area of 43.2 cm x 43.2 cm with 42 cm high walls (Med Associates, Inc., St. Albans, VT, USA), under very low light (< 5 lux). Animals were placed at the center of the novel chamber and allowed to explore for 15 minutes while vertical counts and ambulatory distance were tracked and recorded using infrared beams (Pandey et al., 2019).

Light-Dark Box

The light-dark box was the same activity chamber as used for novelty-induced locomotor activity, but with a two-chamber light-dark insert (Med Associates, Inc., St. Albans, VT, USA). The light chamber was illuminated by LED lamp at 400 lux in the center. Animals were acclimated to the light-dark box in one 5-minute session on the day before testing. On test days, animals were placed in the corner of the light chamber, facing away from the dark chamber, and allowed to explore the light-dark box for 5 minutes, while time spent in the light chamber and number of entries into the light chamber were tracked and recorded using infrared beams. Research suggests that behavior in the light-dark box does not significantly change on repeat testing (Holmes et al., 2001; Onaivi & Martin, 1989).

Open Field

The open field was an arena of 81 cm x 81 cm with 31.5 cm high walls (Med Associates, Inc., St. Albans, VT, USA). Testing was conducted under ambient room light of 200 lux. Animals were acclimated to the open field in one 10-minute session on the day before testing. On test days, animals were placed at the center of the open field and allowed to explore for 10 minutes, while time in the center area, number of entries into the center, and overall ambulatory distance were tracked and recorded using infrared beams. Research suggests that behavior in an open field does not significantly change on repeat testing (Bronstein, 1972).

Histological analysis

Rats were anesthetized with isoflurane, sacrificed by rapid decapitation, and their brains extracted. Following fixation in 4% paraformaldehyde, cryoprotection in 30% sucrose, and storage at -80 °C, the brains were sliced as 30 μm coronal sections. Microscopic examination was used to ensure that injections were made into the NAc shell and determine the level at which this occurred. Bregma +2.76 – +1.80 mm was considered to be rostral and bregma +1.68 – 0.72 mm was considered caudal. Microinjection sites are shown in **Figure 1B**.

Statistical analysis

Statistical analysis of the FSCV data was conducted using GraphPad Prism 8 (GraphPad Software, La Jolla, CA, USA). Baseline dopamine release and uptake data were analyzed using paired two-tailed Student's *t*-

tests, as this was a within-subject design. The U50,488 concentration response curves were analyzed using a repeated measures (RM) two-way analysis of variance (ANOVA). The dependent variable was [DA]/p and the independent variables were rostral/caudal subregions of NAc shell and U50,488 concentration. All ANOVAs were followed up by Sidak's pairwise *post-hoc* analysis. Analysis of behavioral data was conducted using IBM SPSS Statistics version 24 (IBM, Armonk, NY, USA). Because differences were detected between the subregions using FSCV, an *a priori* decision was made to separately test each subregion. Thus, novelty-induced locomotor activity was analyzed using unpaired, two-tailed *t*-tests and light-dark box and open field behavior were analyzed using paired two-tailed *t*-tests. All data are reported as mean ± standard error of the mean. The significance level for all statistical measures was set at $p < 0.05$.

RESULTS

Experiment 1: Effects of rostro-caudal kappa-opioid stimulation on dopamine dynamics

To determine the effects of KOR stimulation on dopamine dynamics in the rostral compared to caudal NAc shell, dopamine release under baseline conditions and in the presence of U50,488 was measured in male and female rats ($n = 5$/group) using *ex vivo* FSCV. After obtaining stable baseline levels of dopamine release, cumulative concentrations of U50,488 were bath applied to the rostral and caudal NAc shell-containing slices. In females, comparison of dopamine release between rostral and caudal NAc shell at baseline (pre-drug exposure) revealed no significant differences [$t(4) = 1.25$; $p = 0.280$] (**Figure 2A**). Similarly, comparison of dopamine uptake rate (Vmax) between the two subregions also did not reveal significant differences [$t(4) = 0.96$; $p = 0.391$] (**Figure 2B**). On the other hand, for KOR-activation mediated inhibition of dopamine release, a repeated-measures two-way ANOVA comparison of the rostral and caudal subregions of the NAc shell revealed main effects of U50,488 concentration [$F(4, 32) = 74.89$, $p < 0.0001$] and NAc subregion [$F(1, 8) = 5.96$, $p < 0.05$], as well as a significant interaction between U50,488 concentration and subregion [$F(4, 32) = 2.93$, $p < 0.05$]. *Post-hoc* Sidak's pairwise comparisons revealed that U50,488 at the 0.3 and 1.0 μM concentrations inhibited dopamine release to a significantly greater extent in the caudal compared to rostral NAc shell ($p < 0.05$; **Figure 2C**). The maximal effect of U50,488-induced inhibition of dopamine release was significantly greater in the caudal compared to rostral subregion of the NAc shell [$t(4) = 8.246$; $p < 0.01$] (**Figure 2D**). A paired *t*-test comparison of the EC50 between rostral and caudal subregions also showed a significant difference [$t(4) = 7.26$, $p < 0.01$] (**Figure 2E**). In males, comparison of dopamine release between the rostral and caudal NAc shell at baseline (pre-drug exposure) also revealed no significant differences [$t(4) = 0.28$; $p = 0.796$] (**Figure 2F**). Similarly,

comparison of dopamine uptake rate (Vmax) between rostral and caudal NAc shell at baseline did not reveal significant differences [$t(4) = 0.56$; $p = 0.605$] (**Figure 2G**). On the other hand, and similar to females, for KOR-activation mediated inhibition of dopamine release, a repeated-measures two-way ANOVA comparison of rostral and caudal subregions of the NAc shell revealed main effects of U50,488 concentration [$F(4, 32) = 121.3$, $p < 0.0001$] and subregion [$F(1, 8) = 12.02$, $p < 0.01$], as well as a significant interaction between U50,488 concentration and subregion [$F(4, 32) = 7.04$, $p < 0.001$]. *Post-hoc* Sidak's pairwise comparisons revealed that U50,488 at the 0.10 μM ($p < 0.01$), 0.3 μM ($p < 0.001$), and 1.0 μM ($p < 0.001$) concentrations inhibited dopamine release to a significantly greater extent in the caudal compared to rostral NAc shell (**Figure 2H**). The maximal effect of U50,488-induced inhibition of dopamine release was significantly greater in the caudal compared to rostral subregion of the NAc shell [$t(4) = 4.88$; $p < 0.01$] (**Figure 2I**). A paired *t*-test comparison of the EC50 between rostral and caudal regions also showed a significant difference [$t(4) = 4.96$, $p < 0.01$] (**Figure 2J**). Direct comparison between males and females revealed no significant sex differences in dopamine kinetics, KOR function, efficacy or potency. These data indicate that, in both males and females, while KOR activation inhibits dopamine release in both the rostral and caudal subregions of NAc shell, the effect is greater in the caudal subregion. Furthermore, this effect appears to be driven by a difference in both efficacy, shown by greater maximal effect in inhibition of dopamine release, and by potency, demonstrated by a reduction in EC50.

Experiment 2: Effects of rostro-caudal kappa-opioid stimulation on novelty-induced locomotor activity

To determine the effects of KOR stimulation on novelty-induced locomotor activity, rats ($N = 33$) were tested in a novel activity chamber following injection in the rostral or caudal NAc shell with U50,488 or saline vehicle (between-subject). While unpaired *t*-tests revealed that U50,488 compared to saline vehicle had no significant effect on vertical counts when injected in the rostral shell [$t(19) = -0.58$, $p = 0.572$], it significantly increased them when injected in the caudal shell [$t(19) = -2.92$, $p < 0.01$] (**Figure 3A**). Examination of ambulatory distance revealed that U50,488 compared to saline again had no significant effect in the rostral shell [$t(19) = 0.36$, $p = 0.727$] but significantly reduced ambulatory distance when injected in the caudal shell [$t(19) = 4.26$, $p < 0.01$] (**Figure 3B**). These results demonstrate that U50,488 increases rearing behavior and decreases distance traveled in a novel activity chamber when injected into the caudal but not rostral NAc shell.

Experiment 3: Effects of rostro-caudal kappa-opioid stimulation on behavior in a light-dark box

To determine the effects of KOR stimulation on anxiety-like or avoidance behavior, rats (N = 17) were tested in a light-dark box following injection in the rostral or caudal NAc shell with U50,488 or saline vehicle (within-subject). Paired t-tests on time spent in the light chamber revealed that U50,488 compared to saline vehicle led to a non-significant increase following injection in the rostral shell (+17%, [$t(10) = -1.55$, $p = 0.152$]) and a significant reduction following injection in the caudal shell [$t(5) = 2.93$, $p < 0.05$] (**Figure 4A**). Examination of number of entries into the light chamber revealed no significant effects with injection in either the rostral shell [$t(10) = 1.05$, $p = 0.317$] or the caudal shell [$t(5) = 1.01$, $p = 0.321$] (**Figure 4B**). These results suggest that U50,488 induces avoidance behavior in a light-dark box when injected into the caudal but not rostral NAc shell.

Experiment 4: Effects of rostro-caudal kappa-opioid stimulation on behavior in an open field

To determine the effects of KOR stimulation on another measure of anxiety-like or avoidance behavior as well as general locomotor activity, rats (N = 20) were tested in an open field following injection in the rostral or caudal NAc shell with U50,488 or saline vehicle (within-subject). Paired t-tests on time spent in the center area revealed that U50,488 compared to saline vehicle had no significant effects when injected in either the rostral shell [$t(11) = 0.73$, $p = 0.478$] or the caudal shell [$t(6) = 1.12$, $p = 0.306$] (**Figure 5A**). However, examination of number of entries into the center revealed that U50,488 compared to saline vehicle led to a significant increase in the number of center entries following injection in the rostral shell [$t(11) = -3.35$, $p < 0.01$] and to a non-significant decrease when injected in the caudal shell (-24%, [$t(6) = 0.56$, $p = 0.596$]) (**Figure 5B**). Examination of ambulatory distance revealed that U50,488 compared to saline vehicle non-significantly increased ambulatory distance following injection in the rostral shell (+18%, [$t(11) = -1.40$, $p = 0.189$]) and significantly reduced it following injection in the caudal shell [$t(6) = 4.22$, $p < 0.01$] (**Figure 5C**). These results suggest that U50,488 reduces avoidance behavior in an open field when injected into the rostral shell and reduces locomotor activity when injected into the caudal shell.

DISCUSSION

Here, we show that activation of KORs across the rostro-caudal axis of the NAc shell results in different and sometimes opposite effects on dopamine release and behavior. While KOR activation throughout the shell

inhibits dopamine release, it does so to a significantly greater extent in the caudal compared to the rostral subregion. Moreover, activation of KORs in the caudal shell increases rearing behavior and anxiety-like or avoidance behavior, and reduces locomotor activity, while activation in the rostral shell instead reduces avoidance behavior. These data suggest that KOR activation in the NAc shell leads to different affective behaviors depending on its specific location, possibly due to its differential level of dopamine inhibition.

While KOR activation with U50,488 inhibited electrically stimulated dopamine release across the NAc shell, this effect was greater in the caudal compared to the rostral shell. The regionally differential effect appears to be a result of increased potency as well as efficacy in the caudal shell. As dopamine transmission itself was equivalent in the two subregions, as measured by dopamine kinetics, it appears unlikely that the KOR-induced differences in dopamine release were due to different degrees of dopamine innervation. Indeed, studies with tract tracing have shown that the mesolimbic projection is equally dense across the subregions, although the projection neurons themselves may be genetically distinct (Poulin et al., 2018). Interactions between dopamine and other neuromodulatory neurochemicals could also contribute to differences in dopamine measurements across the rostro-caudal axis (Gill & Grace, 2011; Park et al., 2010; Richard & Berridge, 2011). It is unlikely that the KOR receptor is structurally different between the subregions. Nevertheless, the affinity of the agonist to the receptor appears to be greater in the caudal shell, as shown by a reduction in EC50 of U50,488. Earlier work with cholecystokinin has similarly found subregion-specific effects of this neuropeptide on levels of dopamine, despite the presumed presence of cholecystokinin receptors in both the rostral and caudal NAc (Ruggeri et al., 1987). Ligand affinity can be influenced by many factors, including cellular milieu and properties of the lipid bilayer, which alter G-protein coupling (Xu et al., 2006; Yan et al., 2008). Therefore, the intracellular cascade triggered by U50,488 binding may itself be different along the rostro-caudal axis of the NAc shell.

In the caudal NAc shell, KOR activation not only significantly reduced electrically stimulated dopamine release, but also increased rearing behavior in a novel activity chamber and anxiety-like or avoidance behavior in a light-dark box, and reduced locomotor activity in both a novel activity chamber and a familiar open field. Increased rearing in a novel environment has been associated with reduced levels of dopamine in the striatum (Abdurakhmanova et al., 2019) and, in ethanol-naïve rats, predicts subsequent high-level ethanol drinking (Pandey et al., 2019). Thus, we hypothesize that the novelty-induced rearing may represent increased risk-taking or impulsivity. Increased anxiety-like behavior, as measured in an elevated plus maze, has been associated with reduced dopamine release and activity in the NAc shell (Avena et al., 2008; Razavi et al., 2014), and the ability of the KOR to induce anxiety-like or avoidance behavior in the present study is in line with prior observations of its aversive effects in the caudal NAc shell (Castro &

Berridge, 2014). Finally, while the effect of dopamine on locomotor activity is commonly thought to involve the NAc core, a reduction of dopamine with application of 7-OH-DPAT in the NAc shell has also been found to decrease locomotor activity (Clements & Greenshaw, 2005). Thus, the KOR-induced reduction of dopamine release in the caudal NAc shell could be responsible for the array of behavioral changes observed in the present study.

In the rostral NAc shell, while KOR activation reduced electrically stimulated dopamine release to a lesser extent than in the caudal shell, it in fact reduced anxiety-like or avoidance behavior, as measured by a slight increase in time in the light chamber of a light-dark box and a significant increase in the number of center entries in an open field. This demonstrates that U50,488 in the rostral shell induces behavioral effects and positive affective reactions, consistent with prior observations of its positive hedonic effects (Castro & Berridge, 2014). The findings of subregion-specific KOR-induced differences in *degree* for dopamine release but *direction* for behavior may parallel effects of corticotropin-releasing factor, where, for example, central injections of this neuropeptide at low doses induce taste preference but at high doses induce aversion (Heinrichs et al., 1991). Therefore, the diminished inhibition of dopamine release in the caudal shell could be responsible for promoting positive affect.

Together, the data indicate that KOR stimulation across the rostro-caudal axis of the NAc shell results in different levels of inhibition of dopamine release and either positive or negative affective behavior. While direct tests remain to be conducted to demonstrate that these changes in behavior are *caused* by changes in dopamine dynamics, the data nevertheless demonstrate that the anatomical heterogeneity of the NAc shell extends to differences in the effects of KOR stimulation on both dopamine and affective behavior.

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FIGURE LEGENDS

Figure 1: Localization of FSCV and microinjections. (A) Carbon-fiber recording electrode placement locations in rostral and caudal subregions of the NAc shell in female and male rats (n = 5/group; red, female rostral; green, male rostral; purple, female caudal; blue, male caudal). (B) Microinjection locations for behavior studies (green dots, male rostral; blue dots, male caudal). For novelty-induced locomotor activity (Experiment 2), 21 rats received injections in the rostral NAc shell (n = 9 – 12/treatment) and 12 received them in the caudal NAc shell (n = 6/treatment). For light/dark box testing (Experiment 3), 11 rats received injections in the rostral NAc shell and 6 received them in the caudal NAc shell. For open field testing (Experiment 4), 13 rats received injections in the rostral NAc shell and 7 received them in the caudal NAc shell.

Figure 2: Stimulated dopamine release and impact of KOR activation on dopamine in the rostral and caudal NAc shell. Panels A-E represent data in females. (A) There was no significant difference in baseline dopamine release in rostral (red bar) compared to caudal (purple bar) accumbens shell. (B) Dopamine uptake rate was also not different between the rostral and caudal accumbens shell. (C) Function of KOR was significantly greater in the caudal versus rostral accumbens shell. (D) Maximal KOR-mediated reduction in dopamine release was significantly greater in the caudal compared to rostral accumbens shell. (E) Potency of KORs was greater in the caudal compared to rostral accumbens shell. Panels F-J represent data in males. (F) There was no significant difference in baseline dopamine release in rostral (green bar) compared to caudal (blue bar) accumbens shell. (G) Dopamine uptake rate did not differ between the rostral and caudal accumbens shell. (H) Function of KOR was greater in the caudal versus rostral accumbens shell. (I) Maximal KOR-mediated reduction in dopamine release was significantly greater in the caudal compared to rostral accumbens shell. (J) Potency of KORs was greater in the caudal compared to rostral accumbens shell. EC50, concentration of drug that results in the half-maximal response in the tissue; KOR, kappa opioid receptor; NAc, nucleus accumbens; U50,488, KOR agonist. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

Figure 3: Impact of KOR activation in the rostral and caudal NAc shell on behavior in a novel activity chamber. (A) Stimulation of KORs in the caudal but not rostral accumbens shell increased rearing behavior in a novel activity chamber, measured as vertical counts. (B) Stimulation of KORs in the caudal but not rostral accumbens shell reduced distance traveled in a novel activity chamber, measured as ambulatory distance. KOR, kappa opioid receptor; NAc, nucleus accumbens; U50,488, KOR agonist. ** $p < 0.01$.

Figure 4: Impact of KOR activation in the rostral and caudal NAc shell on behavior in a light-dark box. (A) Stimulation of KORs in the caudal but not rostral accumbens shell reduced time spent in the light chamber of a light-dark box. (B) Stimulation of KORs in neither the rostral nor the caudal accumbens shell affected the number of entries into the light chamber of a light-dark box. KOR, kappa opioid receptor; NAc, nucleus accumbens; U50,488, KOR agonist. * $p < 0.05$.

Figure 5: Impact of KOR activation in the rostral and caudal NAc shell on behavior in an open field. (A) Stimulation of KORs in neither the rostral nor the caudal accumbens shell affected the time spent in the center of an open field. (B) Stimulation of KORs in the rostral but not caudal accumbens shell increased the number of entries into the center of an open field. (C) Stimulation of KORs in the caudal but not rostral accumbens shell reduced distance traveled in an open field. KOR, kappa opioid receptor; NAc, nucleus accumbens; U50,488, KOR agonist. ** $p < 0.01$.

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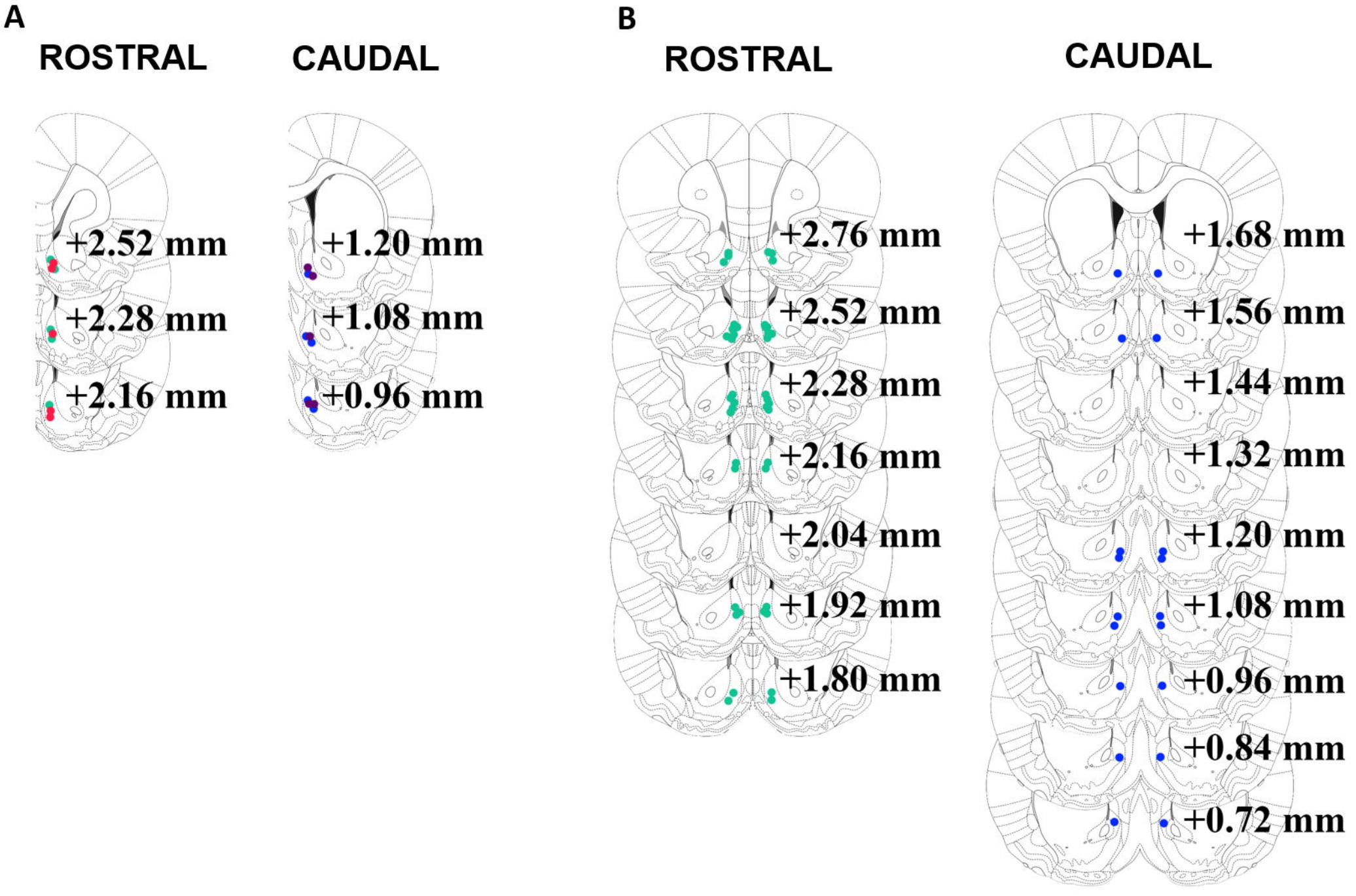
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CAUDAL
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+2.16 mm
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+1.08 mm
+0.96 mm
B
ROSTRAL
CAUDAL
+2.76 mm
+2.52 mm
+2.28 mm
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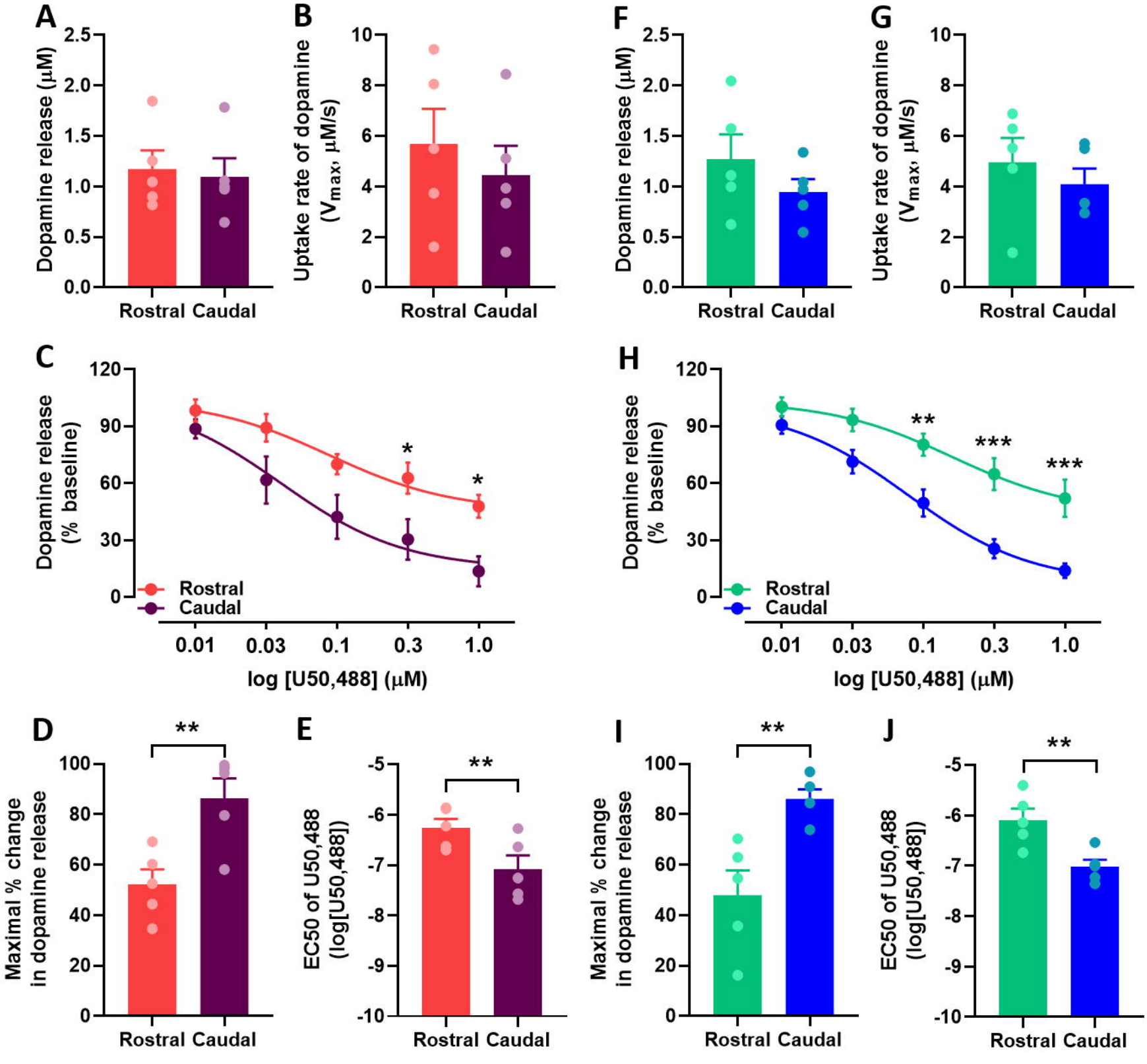
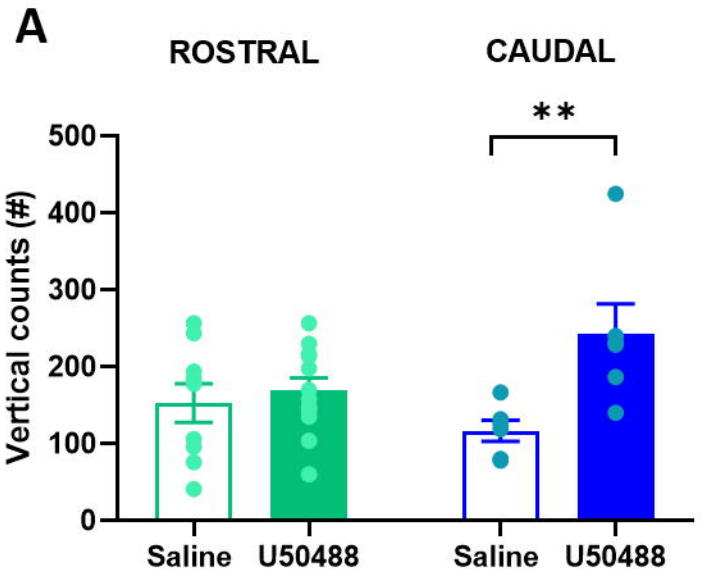

A
Dopamine release (μM)
2.5
2.0
1.5
1.0
0.5
0.0
Rostral Caudal
B
Uptake rate of dopamine (V_{max}, μM/s)
10
8
6
4
2
0
Rostral Caudal
F
Dopamine release (μM)
2.5
2.0
1.5
1.0
0.5
0.0
Rostral Caudal
G
Uptake rate of dopamine (V_{max}, μM/s)
10
8
6
4
2
0
Rostral Caudal
C
Dopamine release (% baseline)
120
90
60
30
0
*
*
Rostral
Caudal
0.01 0.03 0.1 0.3 1.0
log [U50,488] (μM)
H
Dopamine release (% baseline)
120
90
60
30
0
**
***
***
Rostral
Caudal
0.01 0.03 0.1 0.3 1.0
log [U50,488] (μM)
D
**
Maximal % change in dopamine release
100
80
60
40
20
0
Rostral Caudal
E
**
EC50 of U50,488 (log[U50,488])
-5
-6
-7
-8
-9
-10
Rostral Caudal
I
**
Maximal % change in dopamine release
100
80
60
40
20
0
Rostral Caudal
J
**
EC50 of U50,488 (log[U50,488])
-5
-6
-7
-8
-9
-10
Rostral Caudal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CAUDAL
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100
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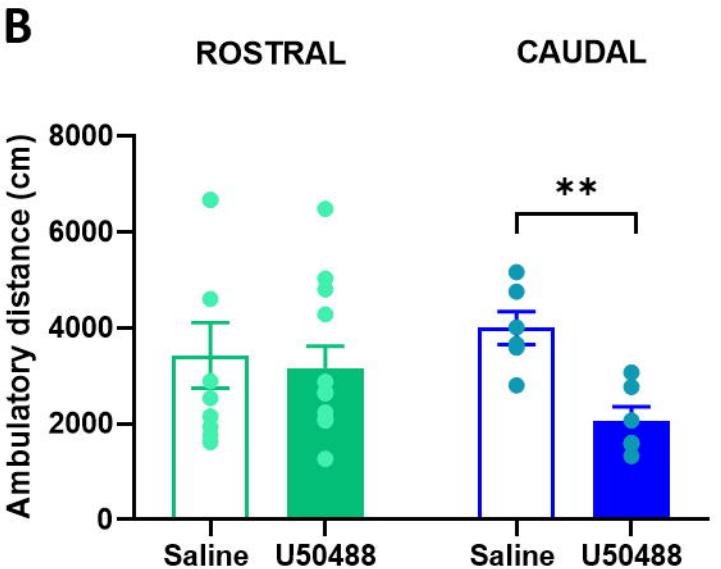
U50488
Saline
U50488


B
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CAUDAL
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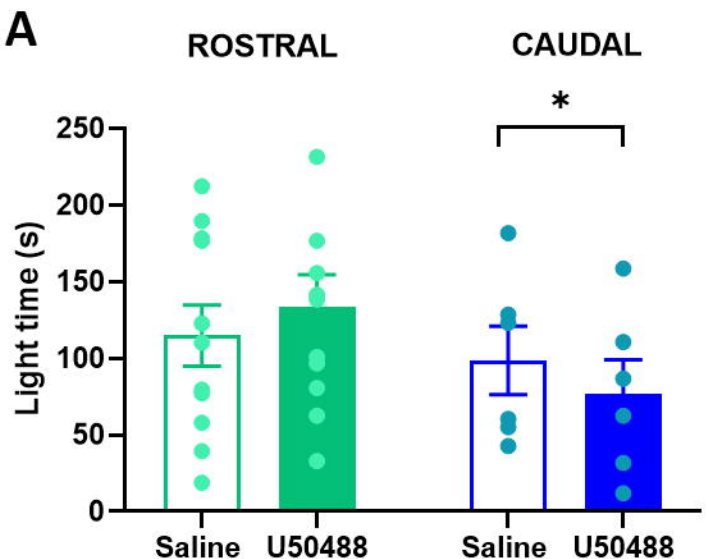
4000
2000
0
Saline
U50488
Saline
U50488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CAUDAL
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Light time (s)
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50
0
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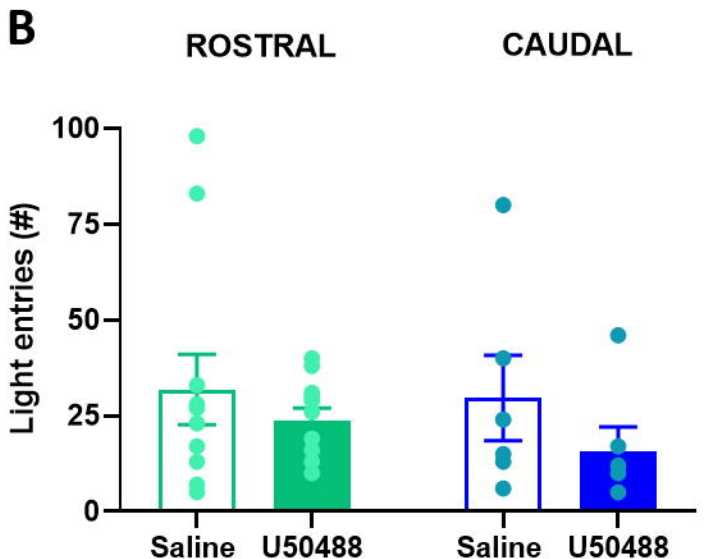
U50488
Saline
U50488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Light entries (#)
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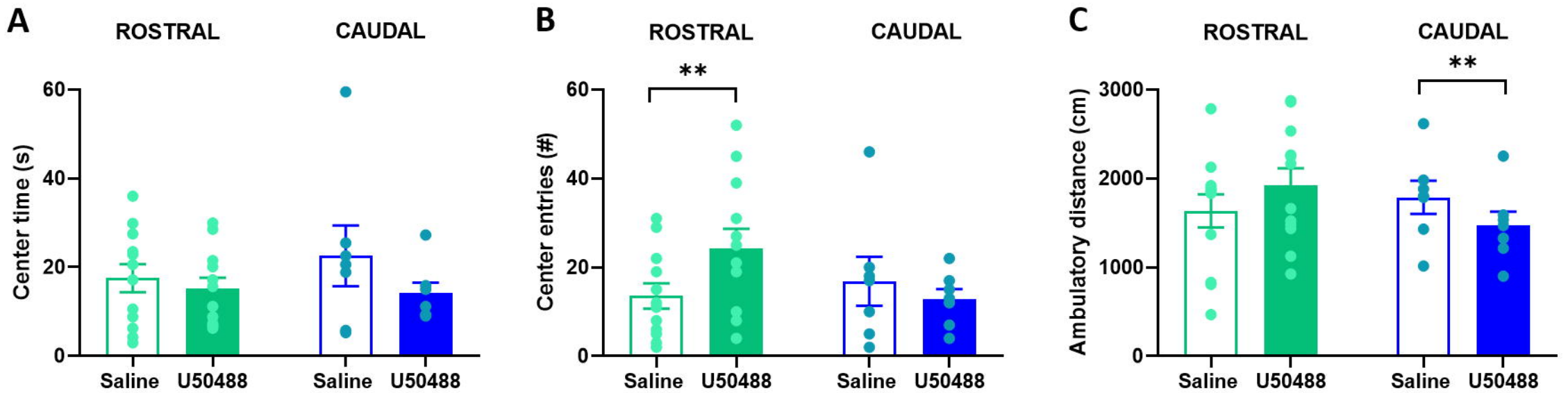
Saline
U50488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CAUDAL
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20
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Saline
U50488
Saline
U50488
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ROSTRAL
CAUDAL
**
Center entries (#)
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40
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0
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U50488
Saline
U50488
C
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CAUDAL
**
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