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Study of activity and connectivity of the *Superior Colliculus* in pharmacological animal models of Schizophrenia

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ABSTRACT

Schizophrenia is a severe mental illness characterized by positive symptoms (hallucinations, delusions, aberrant thoughts), negative symptoms (depression, social isolation), and cognitive impairments, particularly in executive functions and working memory. Recent studies suggest that subcortical regions involved in sensory processing may also be affected in early stages of the disease. Specifically, the superior colliculus (SC)-pulvinar-amygdala circuit has been proposed as a potential contributor to visual processing deficits in schizophrenia.

This study was aimed at investigating alterations in SC activity in animal models of schizophrenia induced by non-competitive antagonists of NMDA glutamate receptors (NMDA-Rs), such as phencyclidine (PCP), dizocilpine (MK-801), or ketamine, via *in-situ* hybridization studies of *c-fos* gene, a surrogate marker of neuronal activity. Since we recently described that the behavioral motor syndrome induced by NMDA-R antagonists is dependent on GluN2C subunit, we extended the study of *c-fos* gene expression in GluN2C knockout (GluN2CKO) mice.

Results showed a significant increase *c-fos* gene expression in the lateral part of the SC after MK-801 treatment. This effect was also observed after the administration of PCP and Ketamine, although it did not reach statistical significance. No main effects of genotype or sex were observed.

Overall, we have identified, for the first time, that lateral SC activity is altered in animal models of schizophrenia. These results will have a follow up investigating lateral SC connectivity with the rest of the brain through fMRI studies and provide a better understanding of the neurobiological mechanisms underlying schizophrenia, which may open new diagnostic and treatment possibilities.

RESUM

L'esquizofrènia és una malaltia mental greu caracteritzada per símptomes positius (al·lucinacions, deliris, pensaments aberrants), símptomes negatius (depressió, aïllament social) i deteriorament cognitiu, especialment en funcions executives i memòria de treball. Estudis recents suggereixen que les regions subcorticals involucrades en el processament sensorial també poden veure's afectades en les etapes primerenques de la malaltia. Específicament, s'ha proposat que el circuit format pel col·licle superior (SC), el nucli pulvinar i l'amígdala podria contribuir als dèficits en el processament visual en l'esquizofrènia.

Aquest estudi va tenir com a objectiu investigar les alteracions en l'activitat del SC en models animals d'esquizofrènia induïts per antagonistes no competitius dels receptors NMDA de glutamat (NMDA-R), com fenciclidina (PCP), dizocilpina (MK-801) o ketamina, mitjançant estudis d'hibridació *in situ* del gen *c-fos,* com a marcador d'activitat neuronal. Donat que recentment hem descrit que el síndrome motor conductual induït per antagonistes NMDA-R depèn de la subunitat GluN2C, vam ampliar l'estudi de l'expressió del gen c-fos en ratolins deficients en GluN2C (GluN2CKO).

Els resultats van mostrar un augment significatiu en l'expressió del gen c-fos en la part lateral del SC després del tractament amb MK-801. Aquest efecte també es va observar després de l'administració de PCP i ketamina, tot i que no va assolir significança estadística. No es van observar efectes principals del genotip ni del sexe.

En general, hem identificat per primera vegada que l'activitat del SC lateral està alterada en models animals d'esquizofrènia. Aquests resultats seran ampliats amb estudis de ressonància magnètica funcional (fMRI) per investigar la connectivitat del SC lateral amb la resta del cervell, la qual cosa proporcionarà una millor comprensió dels mecanismes neurobiològics subjacents a la malaltia i pot obrir noves alternatives de diagnòstic i tractament de la mateixa.

RESUMEN

La esquizofrenia es una enfermedad mental grave caracterizada por síntomas positivos (alucinaciones, delirios, pensamientos aberrantes), síntomas negativos (depresión, aislamiento social) y deterioro cognitivo, especialmente en funciones ejecutivas y memoria de trabajo. Estudios recientes sugieren que las regiones subcorticales involucradas en el procesamiento sensorial también pueden verse afectadas en las etapas tempranas de la enfermedad. Específicamente, se ha propuesto que el circuito formado por el colículo superior (SC), el núcleo pulvinar y la amígdala podría contribuir a los déficits en el procesamiento visual en la esquizofrenia.

Este estudio tuvo como objetivo investigar las alteraciones en la actividad del SC en modelos animales de esquizofrenia inducidos por antagonistas no competitivos de los receptores de glutamato NMDA (NMDA-R), como fenciclidina (PCP), dizocilpina (MK-801) o ketamina, mediante estudios de hibridación *in situ* del gen *c-fos,* como marcador de actividad neuronal. Dado que recientemente hemos descrito que el síndrome motor conductual inducido por antagonistas de los receptores de NMDA depende de la subunidad GluN2C, extendimos el estudio de la expresión del gen *c-fos* en ratones deficientes en GluN2C (GluN2CKO).

Los resultados mostraron un aumento significativo en la expresión del gen *c-fos* en la parte lateral del SC después del tratamiento con MK-801. Este efecto también se observó después de la administración de PCP y ketamina, aunque no alcanzó significancia estadística. No se observaron efectos principales del genotipo ni del sexo.

En conclusión, hemos identificado por primera vez que la actividad del SC lateral se altera en modelos animales de esquizofrenia. Estos resultados se complementarán con estudios de resonancia magnética funcional (fMRI) para investigar la conectividad del SC lateral con el resto del cerebro, lo cual proporcionará una mejor comprensión de los mecanismos neurobiológicos subyacentes a la enfermedad y puede abrir nuevas alternativas de diagnóstico y tratamiento de la misma.

1. INTRODUCTION

Schizophrenia is a severe chronic mental illness with a worldwide prevalence of approximately 1% (1). Although its cause is unknown, it seems to rely on an interaction of genetic and environmental factors that alter the brain at a structural, functional, and neurochemical level. It usually begins in early adulthood and includes positive symptoms (e.g., hallucinations, paranoia, psychomotor agitation), negative symptoms (e.g., social withdrawal, apathy, motivational deficits), and cognitive symptoms (e.g., deficits in executive functioning, working memory, and attention). (2)

From a neurochemical point of view, there is no simple hypothesis of schizophrenia involving a single transmitter dysfunction in the brain. To date, several alterations in dopaminergic (3) serotoninergic (4), GABAergic and glutamatergic neurotransmission (5) have been described, and it is now considered that schizophrenia affects different transmitter pathways in different brain regions, which results in impaired circuit function that is responsible for the multiple set of symptoms observed.

One of the most currently used animal models of the disease is based on the glutamatergic hypothesis. This hypothesis postulated a glutamate-mediated deficiency in excitatory neurotransmission via N-methyl-D-aspartate (NMDA) glutamate receptors (NMDA-R). It arose from the clinical observation that sub-anaesthetic doses of non-competitive NMDA-R antagonists, such as phencyclidine (PCP) or ketamine, induced psychotic reactions in healthy individuals and exacerbated symptomatology in schizophrenic patients. In rodents, non-competitive NMDA-R antagonists induce a behavioral motor syndrome that has been considered a correlate of psychotic symptoms since it is reversed with antipsychotic drugs. (6) (7)

In the last decades, pathological mechanisms underlying schizophrenia focused on deficits in cortical brain regions. These abnormalities suggest a neuronal disorganization characteristic of an interruption of neuronal migration during the second trimester of gestation with aberrant patterns of cortical and subcortical connectivity in neuronal connections.

In addition to cortical regions, subcortical regions associated with low-level sensory processing have recently been found to be linked to the pathophysiology of schizophrenia. Rapid visual processing affects these patients with an increased visual masking threshold, which is associated with abnormal structures in the subcortical visual pathways. A classic subcortical pathway responsible for the rapid processing of critical visual information, especially potentially dangerous stimuli, is considered to start in the superior colliculus (SC), pass through the

pulvinar nucleus of the thalamus, and end in the amygdala. A recent study reports that this pathway could be responsible for negative emotion processing. (8)

The SC is the first nucleus in the subcortical pathway to receive information from the retina. It plays a vital role in multisensory integration, spatial attention, and eye movements to control innate behaviors. The most superficial layers of the SC receive afferents from the retinal ganglion cells and the primary visual cortex. In contrast, the intermediate and deeper layers receive afferents from the auditory, somatosensory, and motor cortex and send projections to various subcortical regions, converting the stimuli sensory into actions (Figure 1A) (6) .The functions of the SC include generating saccadic movements, which are the movements that the eyes make to guide their gaze in the direction from which a stimulus comes. It also causes more complex movements involving the head and extremities, such as stopping the movement, running away, or approaching (9). Rodents, like primates, generate rapid, saccadic-like orienting movements of their eyes and possess a laminar organization of the SC that is nearly indistinguishable from primates' SC. (10)

Patients with schizophrenia have impaired subcortical eye movement control, made by the SC and cortically by the frontal eye fields (FEF). The two regions play different roles in eye movement control: the SC is relevant for short-latency saccadic eye movements associated with rapid detection of potentially dangerous stimuli. In contrast, the FEF is associated with target selection. (11)

In addition, evidence of impaired saccadic control in anti-saccade function was found for unaffected first-degree relatives of patients with schizophrenia and non-clinical individuals with high positive schizotypal scores. Therefore, there is evidence that suggests that there are abnormalities in SC saccadic function for both schizophrenic patients and susceptible individuals. This could be useful for the early diagnosis of schizophrenia. (7)

Currently, our understanding of the specific influence of the SC in schizophrenia remains uncertain. While abnormalities in the structure and function of the SC have been observed in individuals with schizophrenia, we still lack a comprehensive understanding of how these changes contribute to the symptoms and characteristics of the disease. Moreover, few studies have investigated the role of the SC in animal models of schizophrenia.

In this study we sought to examine whether a different activity of the SC exist in animal models of schizophrenia. For this purpose, we investigated the expression of *c-fos* gene, a surrogate marker of neuronal activity, in different subdivisions of the SC in mice treated with NMDA-R

antagonists, under the working hypothesis that increased activity in SC would be observed. Moreover, since we have recently demonstrated that GluN2C subunit of NMDA-R appears to be strongly involved in the motor components of the behavioral syndrome induced by non-competitive NMDA-R antagonists, we extended the study to GluN2C knockout (GluN2CKO) mice.

2. METHODS AND MATERIALS

2.1 Subjects

We used 8-12-week-old male C57BL6/J, and GluN2CKO and wild-type mice of both genders. Animals were maintained in the University of Barcelona, UB's animal facilities, in a controlled environment (12-h light/dark cycle, 22 ± 1 ºC) with *ad libitum* access to food and water. Experimental procedures were conducted in accordance with national (Royal Decree 53/2013) and European legislation (Directive 2010/63/EU, on the protection of animals used for scientific purposes, September 22, 2010). The UB Institutional Animal Care and Use Committee approved them.

2.2 Drugs

MK-801 and PCP (Sigma-Aldrich, Natick, MA) were dissolved in saline and injected by intraperitoneal (i.p.) or subcutaneous (s.c.) route, respectively. Ketamine (Ketolar®, Pfizer) was dissolved in saline and injected i.p. Doses (MK-801 0.1 and 0.25 mg/kg; PCP 5 and 10 mg/kg; ketamine 30 mg/kg) are expressed as free bases and shown according to the literature (8,9). The volume of injection was 4 ml/kg in all cases.

2.3 In situ hybridization

The *in-situ* hybridization studies were previously performed in (10,15,16). Briefly, mice were euthanized an hour after the treatment, and their brains were rapidly removed, frozen on dry ice, and stored until processed. Brain sections were cut using a cryostat and mounted onto slides, then fixed and hybridized with a labeled oligonucleotide probe specific for *c-fos* mRNA. The hybridized areas were exposed to several films (Biomax MR films) for seven to ten days. The resulting images were analysed to obtain mean grey values, which were used to quantify *c-fos* expression in different brain regions. In addition, two or three consecutive brain sections at any level of interest (AP coordinates from (14) were analysed for each mouse and averaged to obtain individual values.

2.4 Acquisition and quantification of the images of in-situ hybridization experiments

Images from the following previous experiments of *c-fos* brain expression in animal models of schizophrenia were obtained:

Experiment 1 (17)**:** Study of *c-fos* mRNA expression in C57BL/6J male mice after acute or sub-chronic treatment with PCP (10 mg/kg, sc). Experimental groups: saline-saline (n=4), saline-PCP (n=4), PCP-PCP (n=3). Three duplicates were used.

Experiment 2 (10)**:** Study of *c-fos* mRNA expression in WT and GluN2CKO male mice after acute treatment with PCP (5 mg/kg, sc). Experimental groups: saline-WT (n=5), PCP-WT (n=5), saline-GluN2CKO (n=5), PCP- GluN2CKO (n=5). Two duplicates were used.

Experiment 3 (10)**:** Study of *c-fos* expression in WT and GluN2CKO male mice after acute treatment with MK-801 (0,1 and 0,25 mg/kg, sc). Experimental groups: saline-WT (n=5), MK-801 0,1-WT (n=5), MK-801 0,25-WT (n=5), saline-GluN2CKO (n=5), MK-801 0,1-GluN2CKO (n=5), MK-801 0,25-GluN2CKO (n=5). Two duplicates were used.

Experiment 4 (16)**:** Study of *c-fos* mRNA expression in WT and GluN2CKO male and female mice after acute treatment with Ketamine (30 mg/kg, sc). Experimental groups: male saline-WT (n=5), male Ketamine-WT (n=5), male saline- GluN2CKO (n=5), male Ketamine-GluN2CKO (n=5), female saline-WT (n=6), female Ketamine-WT (n=6), female saline-GluN2CKO (n=6), female ketamine-GluN2CKO (n=6). Two duplicates were used.

Films were correctly labelled and scanned consecutively, and images of coronal sections of the mice brains at coordinate anteroposterior - 4,36 (14) were obtained by using the Micro-manager 14 software. The image files obtained were saved in folders depending on the experiment they came from. Subsequently, four regions of interest were delineated in both cerebral hemispheres (superficial SC, lateral SC, medial SC, and periaqueductal gray matter (PAG), see Figure 1B). During the quantification of the films, an increase in *c-fos* mRNA signal was observed in the periaqueductal gray matter (PAG), so we decided to include the quantification of this area into the study. The PAG is located between the forebrain and the brainstem and plays a role in regulating emotional responses, modulating pain, and generating defensive responses. Dysfunction in the PAG has been associated with various disorders, including those related to pain, anxiety disorders, and emotional regulation. Therefore, we found it interesting to investigate its potential involvement in animal models of schizophrenia.

The Fiji-ImageJ software was used to quantify the expression signal of the *c-fos* mRNA expression in each region of interest. The experimenter was blind to treatment, genotype, and gender conditions during quantification.

A) **B)**

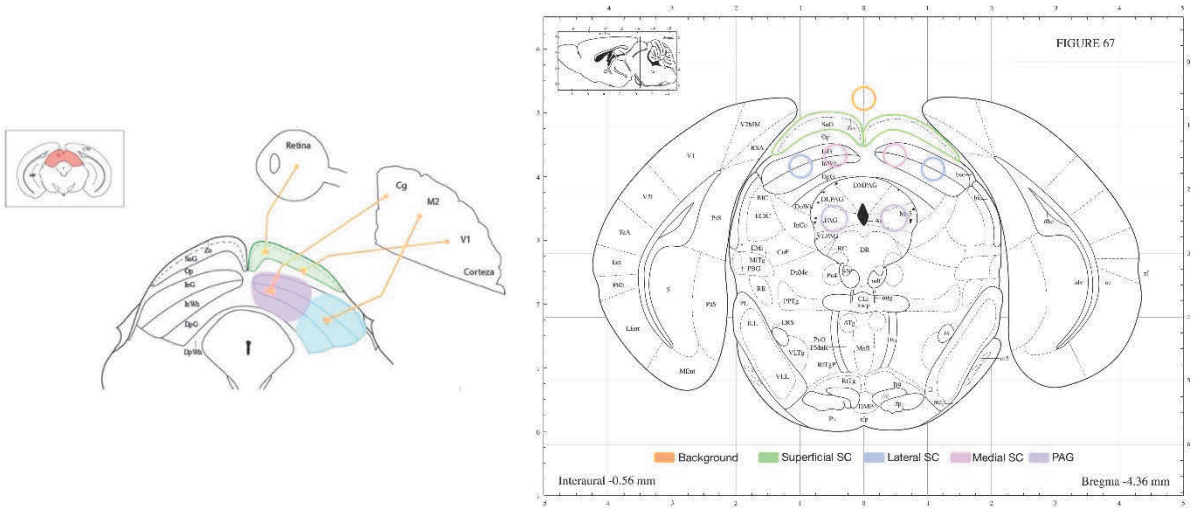

Figure 1. A) Mouse coronal brain section showing the structure of the SC and its primary afferents. *Figure modified by good notes program extracted from* (12) *based on* (9) *and* (13)*. Brain section of Interaural 0.16 mm and Bregma -3.64 mm coordinates obtained from* (14)**.**
B) Representation of the delineation of the regions for the quantification of *c-fos* mRNA signal based on the atlas (14) (Bregma -4.36 mm). Superficial Superior Colliculus (Superficial SC), Lateral Superior Colliculus (Lateral SC), Medial Superior Colliculus (Medial SC), and Periaqueductal gray matter (PAG).

2.5 Statistical analysis

Initially, raw data of signal measured in each area of interest was dumped into Excel tables and sorted according to film number, experimental condition, mouse number, and section number.

After that, the background value was subtracted from each value, and the average of the right- and left-brain sides were calculated, as well as the average signal between duplicates. Then, the mean of the *c-fos* mRNA signal was calculated for each brain region based on the experimental condition.

An additional table was generated where the values were normalized in relation to the control group in each experiment. The final data was expressed as mean ± standard error of the mean (SEM).

Statistical analysis was carried out using GraphPad Prism V.6 software. Before performing the statistical analysis, the normality of the data was checked using the Shapiro-Wilk test.

In experiment 1, a one-way ANOVA (treatment as a factor of variation) was used. In experiments 2-3, a two-way ANOVA (treatment and genotype as factors of variation) was used. In experiment 4, a two-way ANOVA (treatment and genotype as factors of variation) on the lateral SC and PAG, and Mann-Whitney test on the superficial SC and medial SC were used for each genotype. Posterior, Tukey's multiple comparisons test was used to perform *post hoc* comparisons to determine specific differences between the groups. In all cases, the significance level was set at $p < 0.05$.

3. RESULTS

3.1 Experiment 1

The effects of acute and chronic PCP (10 mg/kg) on *c-fos* gene expression in male mice are shown in Figure 2 and the statistical analysis is in Table 1. One-way ANOVA did not show significant differences between groups in all the regions studied.

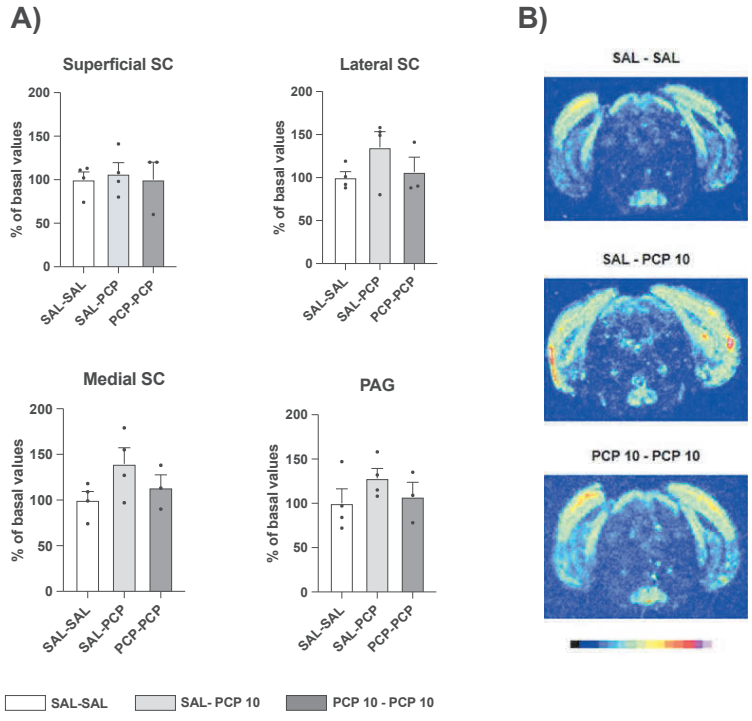

Figure 2. **A)** Effects of acute (SAL-PCP 10) and chronic (PCP 10-PCP 10) administration of PCP (10 mg/Kg, s.c) on *c-fos* expression in male mice. **B)** Representative coronal sections of mice brain (AP: Bregma -4.24 mm to -4.48 mm) showing the effects of NMDA-R treatment on

c-fos expression in each experimental group. SC, superior colliculus; PAG, periaqueductal gray matter.

3.2 Experiment 2

The results of PCP treatment (5 mg/kg) on *c-fos* gene expression in WT and GluN2CKO mice is shown in Figure 3. See table 1 for statistical analysis. For superficial SC data, two-way ANOVA showed a significant main effect of genotype and a significant interaction between genotype and treatment. For lateral SC, two-way ANOVA showed a significant main effect of treatment. However, post-hoc comparisons showed no significant differences between groups in any cases. For medial SC and PAG no significant effects in the two-way ANOVA were found.

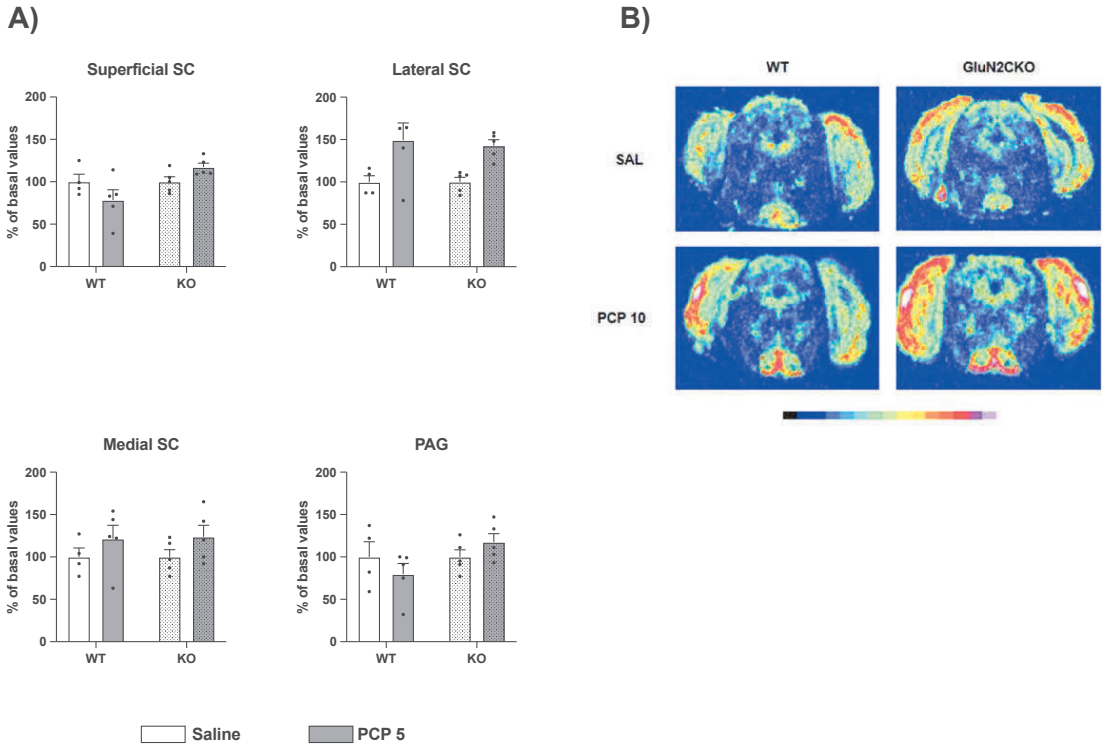

Figure 3. A) Effects of PCP (5 mg/Kg, s.c.) on the expression of *c-fos* gene in male WT and GluN2CKO mice. **B)** Representative coronal sections of mice brain (AP: Bregma -4.36 to -4.60) showing the effects of NMDA-R treatment on *c-fos* expression in each experimental group. SC, superior colliculus; PAG, periaqueductal gray matter.

3.3 Experiment 3

The results of MK-801 treatment (0,1 and 0,25 mg/kg) on *c-fos* gene expression in WT and GluN2CKO mice is shown in Figure 4. See table 1 for statistical analysis. Two-way ANOVA showed a significant main effect of treatment, but no effect of genotype nor interaction, for all

the regions studied. Post-hoc comparisons only showed a significant difference between WT saline and WT MK-801 0,25 groups ($p<0.01$) for lateral SC.

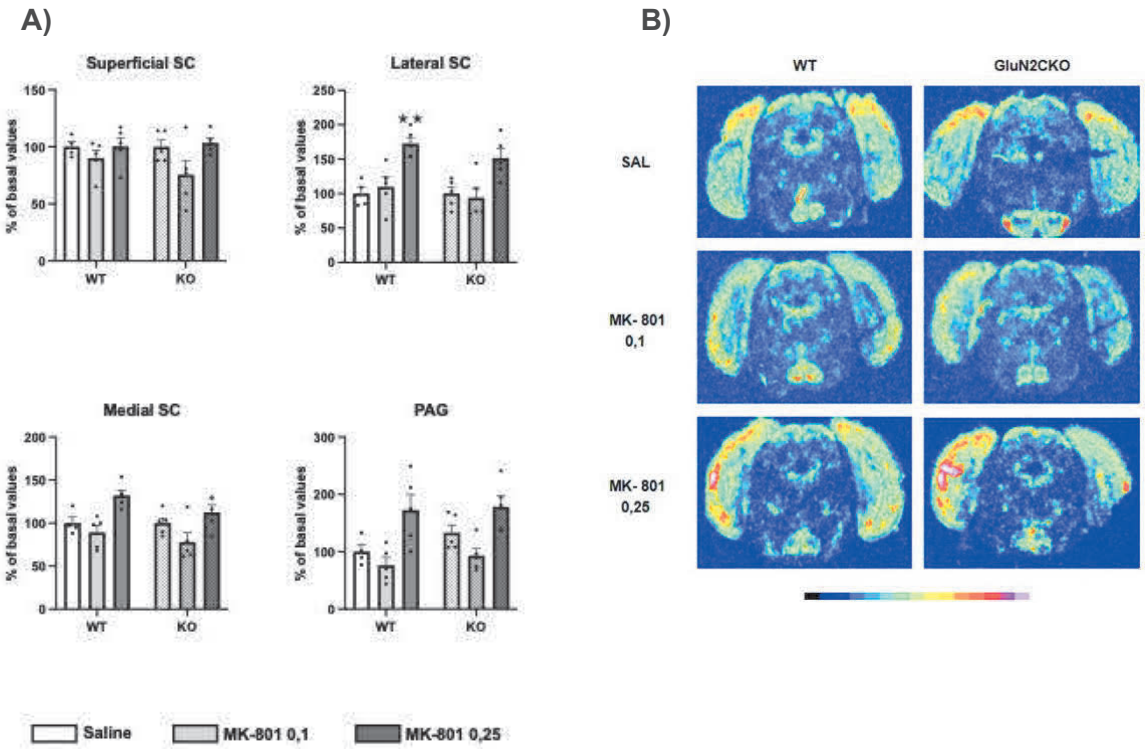

Figure 4. A) Effects of MK-801 (0.1 and 0.25 mg/kg, i.p.) on *c-fos* gene expression in WT and GluN2CKO male mice. *p<0,05, **p<0,01 *vs* saline. **B)** Representative coronal sections of mice brain (AP: Bregma -4.36 to -4.60) showing the effects of NMDA-R treatment on *c-fos* expression in each experimental group. SC, superior colliculus; PAG, periaqueductal gray matter.

3.4 Experiment 4

Figure 5 shows the results of Ketamine treatment (30 mg/kg) on *c-fos* gene expression in male (♂) and female (♀) WT and GluN2CKO mice, respectively. See table 1 for statistical analysis.

In male mice, U-Mann-Whitney test showed no significant effects of treatment nor genotype for superficial SC or medial SC. Additionally, two-way ANOVA showed no significant effect of treatment, genotype nor interaction, for the lateral SC and PAG (Figure 5A and 5B).

In female mice, U-Mann-Whitney test showed no significant effects of treatment nor genotype for superficial SC or medial SC. Additionally, two-way ANOVA showed no significant effect of treatment, genotype nor interaction, for the lateral SC and PAG (Figure 5C and 5D).

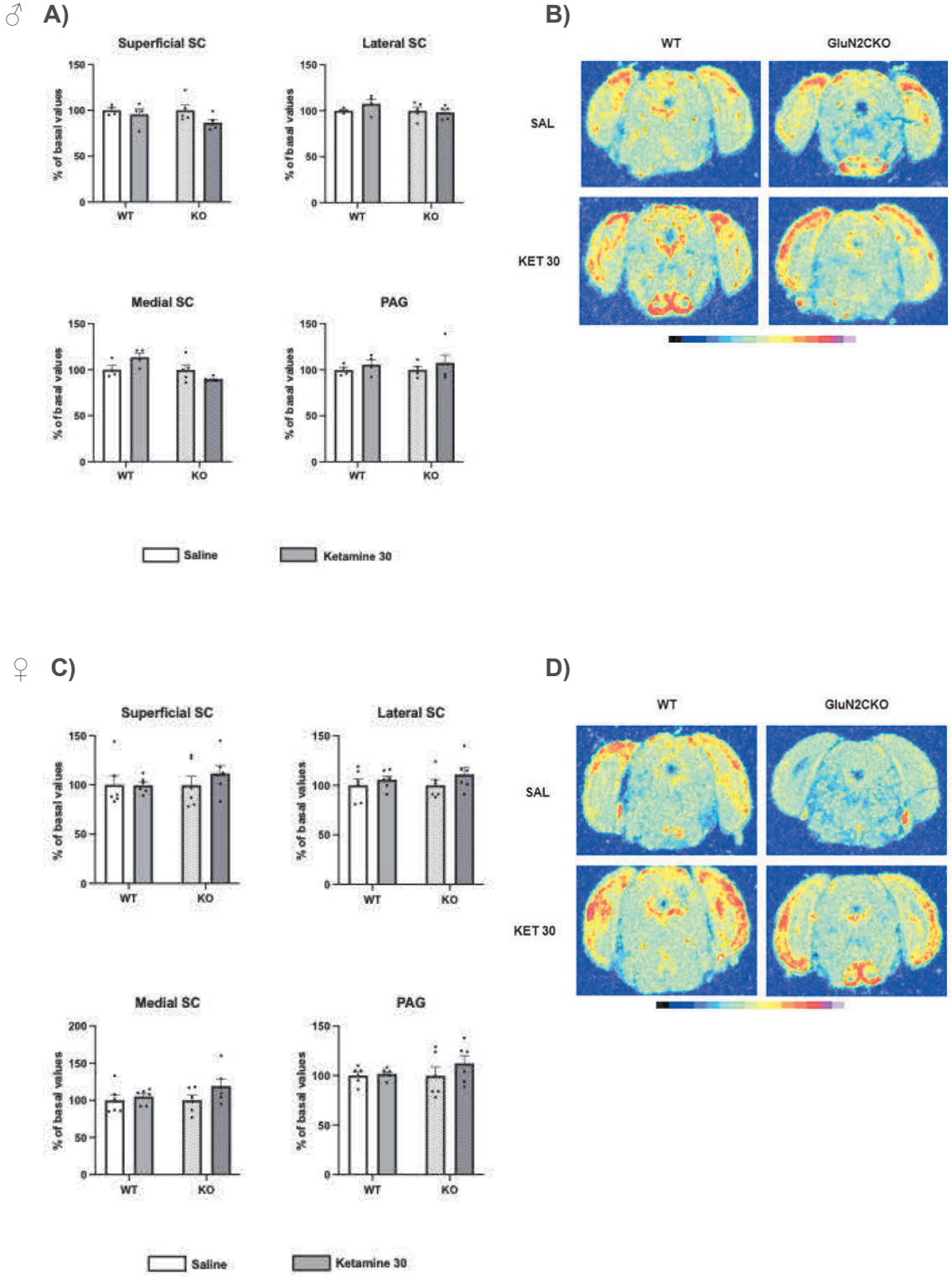

Figure 5. Effects of Ketamine (30 mg/kg, i.p.) on *c-fos* gene expression in WT and GluN2CKO male **(A)** and female **(C)** mice. **B, D)** Representative coronal sections of mice brain (AP: Bregma -4.48 to -4.72 for males, -4.48 to -4.60 for females) showing the effects of NMDA-R treatment on *c-fos* expression in each experimental group. SC, superior colliculus; PAG, periaqueductal gray matter.

4. DISCUSSION

Schizophrenia is a complex psychiatric disorder with neurobiological alterations. Studying subcortical alterations can help us better understand the progression of schizophrenia symptoms and set the foundation for the development of therapeutic strategies.

In this study, we investigated the expression of the *c-fos* gene, a surrogate marker of neuronal activity, in subcortical regions namely the SC and PAG, using *in situ* hybridization studies in mice after the administration of non-competitive NMDA-R antagonists such as PCP, Ketamine and MK-801, which have been extensively used as animal models of the disease. This approach allows the comparison of results done in the same facilities and under the same experimental conditions to obtain better quality results and conclusions.

Interestingly, we have observed an increase in mRNA signal of the *c-fos* gene in the lateral SC after administering MK-801 and a marked tendency for the rest of NMDA-R antagonists. This result shows, for the first time, that there is an increased activity in this area after NMDA-R blockade. Also, a tendency to an increased activity in medial SC and PAG was observed after MK-801 treatment, suggesting a widespread activation of SC by NMDA-R antagonists.

Several factors, such as sample size and/or dose may have influenced the results observed in the different experiments. In experiment 1 we only had three mice available for experimental group, while in the other experiments, 4 to 6 mice were used. Additionally, the dosage used in experiment 2 (PCP 5 mg/kg) could be too low in this test. It appeared that in experiment 3, where the MK-801 was administered, a greater influence of the treatment effect was observed. This could be attributed to the higher affinity of MK-801 as an NMDA receptor antagonist. Therefore, if the dosage of PCP were increased, it is possible that a treatment effect on the sample could be obtained. Moreover, treatment effects in experiment 3 were observed with MK-801 at a dosage of 0.25 mg/kg, whereas no treatment effect was observed with MK-801 at a dosage of 0.1 mg/kg. This indicates that dose-response studies would be more appropriate to reveal the effects of NMDA-R antagonists on *c-fos* expression in SC.

In this study no significant differences were observed in GluN2CKO mice, suggesting that GluN2C subunit of NMDA-R is not crucial for SC activity in animal models of schizophrenia. This result is in accordance with the low expression of this subunit in SC. In fact, the GluN2C subunit is densely expressed in cerebellar granule neurons (18,19) the thalamus (20,21) including the RtN (22,23), and the olfactory bulb.

Additionally, no effects of gender were observed regarding *c-fos* expression in SC and PAG after ketamine treatment. However, further investigation using the other antagonists is needed before a general conclusion is made.

The superior colliculus (SC) is a subcortical structure involved in the generation of saccadic eye movements. There is evidence of impaired express saccades and compromised control of those in individuals with schizophrenia, as well as in first-degree unaffected relatives and nonclinical individuals with higher schizotypy scores. Additionally, a recent study suggests that early visual pathway impairment, as indicated by Visual Evoked Potentials (VEP), could serve as a potential biomarker for cognitive deficits in schizophrenia. These findings suggest that dysfunction in early visual regions may contribute to errors in higher cortical function. In addition, there's evidence that raise the possibility that direct reduction of GABA transmission within the SC itself may be shown in schizophrenia. (24) (25) (11).

Superficial layers of the SC receive information from retinal ganglion cells and the primary visual cortex, while the intermediate and deep layers receive input from the auditory, somatosensory, and motor cortex, and send projections to various subcortical regions, allowing for the transformation of sensory stimuli into actions, for example threatening stimuli (9). The intermediate and deep layers of the SC are divided into the medial superior colliculus (medial SC) and lateral superior colliculus (lateral SC), based on the afferent inputs they receive. The medial SC primarily receives afferents from the cingulate cortex (Cg), while the lateral SC primarily receives afferents from the secondary motor cortex (M2). (13)

On the other hand, the periaqueductal gray (PAG) is connected to the superior colliculus through descending projections. These connections are important for modulating defense responses and pain control. The visual and auditory information processed in the superior colliculus can influence PAG activity, and in turn, the PAG can modulate SC activity. The PAG is also connected to the amygdala, prefrontal cortex, insula, hypothalamus, and hippocampus, and it communicates with structures in the spinal cord to control motor responses. Additionally, the PAG facilitates survival by linking negative emotional states with motivational circuits that are involved in decision-making processes. (26)

Therefore, due to the described different connectivity of medial and lateral SC as well as the PAG, we propose a follow up study to investigate the functional connectivity of these areas with the rest of the brain in animal models of schizophrenia using fMRI studies.

Overall, our results show that there's an alteration of SC activity mainly in its lateral part. That could trigger functional deficits in the cortical level and in the processing of negative emotions in schizophrenia as suggested in recent studies (11). Therefore, it would be of greater interest to investigate behavioral responses related to segregated SC circuits in animal models of schizophrenia such as SC-dependent behavioral responses to unexpected visual stimuli, like the presence of a moving robo-beetle (27) and an unexpected flash of light. (28)

Moreover, further behavioral studies regarding emotional signs and nociceptive threshold in schizophrenia should consider the segregated SC circuits.

5. CONCLUSIONS

In summary, and notwithstanding the limitation of doses and sample size used in the current work, we hypothesize that the lateral SC may be involved in the pathological mechanisms of schizophrenia. Further exploration combining *in situ* hybridization studies with behavioral and fMRI studies can link molecular alterations to functional brain changes of the SC and PAG in schizophrenia, improving our understanding of the disease and the development of new methods for early diagnosis and treatment.

6. ACKNOWLEDGMENTS

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REFERENCES

1. Häfner H, an der Heiden W. Epidemiology of Schizophrenia. The Canadian Journal of Psychiatry. 1997 Mar 1;42(2):139–51.
2. Winship IR, Dursun SM, Baker GB, Balista PA, Kandratavicius L, Maia-de-Oliveira JP, et al. An Overview of Animal Models Related to Schizophrenia. Vol. 64, Canadian Journal of Psychiatry. SAGE Publications Inc.; 2019. p. 5–17.
3. Howes OD, Kapur S. The dopamine hypothesis of schizophrenia: Version III - The final common pathway. Vol. 35, Schizophrenia Bulletin. 2009. p. 549–62.
4. Eggers AE. A serotonin hypothesis of schizophrenia. Med Hypotheses. 2013 Jun;80(6):791–4.
5. Uno Y, Coyle JT. Glutamate hypothesis in schizophrenia. 2019;
6. Isa T, Hall WC. Exploring the Superior Colliculus In Vitro. J Neurophysiol. 2009 Nov;102(5):2581–93.
7. Myles JB, Rossell SL, Phillipou A, Thomas E, Gurvich C. Insights to the schizophrenia continuum: A systematic review of saccadic eye movements in schizotypy and biological relatives of schizophrenia patients. Neurosci Biobehav Rev. 2017 Jan;72:278–300.
8. Waguespack HF, Aguilar BL, Malkova L, Forcelli PA. Inhibition of the Deep and Intermediate Layers of the Superior Colliculus Disrupts Sensorimotor Gating in Monkeys. Front Behav Neurosci. 2020 Dec 22;14.
9. Ito S, Feldheim DA. The mouse superior colliculus: An emerging model for studying circuit formation and function. Vol. 12, Frontiers in Neural Circuits. Frontiers Media S.A.; 2018.
10. Tarrés-Gatius M, López-Hill X, Miquel-Rio L, Castarlenas L, Fabius S, Santana N, et al. Discrimination of motor and sensorimotor effects of phencyclidine and MK-801: Involvement of GluN2C-containing NMDA receptors in psychosis-like models. Neuropharmacology. 2022 Aug 1;213.
11. Shen L, Liu D, Huang Y. Hypothesis of subcortical visual pathway impairment in schizophrenia. Med Hypotheses. 2021 Nov 1;156.
12. Conde-Berriozabal S, García-Gilabert L, García-García E, Sitjà- L, López-Gil J, Muñoz-Moreno E, et al. Altered M2 Cortex-Superior Colliculus visual perception Magnetic Resonance Imaging Core Facility, Institut d'Investigacions August Pi i Sunyer.
13. Savage MA, McQuade R, Thiele A. Segregated fronto-cortical and midbrain connections in the mouse and their relation to approach and avoidance orienting behaviors. Journal of Comparative Neurology. 2017 Jun 1;525(8):1980–99.
14. Paxinos G, Franklin KBJ. The mouse brain in stereotaxic coordinates. Academic Press; 2001.
15. Gatius Tarrés M. Neurobiological mechanisms involved in the antidepressant and psychotomimetic effects of NMDA receptor antagonists: role of the GluN2C subunit. Barcelona; 2019.
16. Tarrés-Gatius M, Miquel-Rio L, Campa L, Artigas F, Castañé A. Involvement of NMDA receptors containing the GluN2C subunit in the psychotomimetic and antidepressant-like effects of ketamine. Transl Psychiatry. 2020 Dec 1;10(1).

17. Castañé A, Santana N, Artigas F. PCP-based mice models of schizophrenia: Differential behavioral, neurochemical and cellular effects of acute and subchronic treatments. Psychopharmacology (Berl). 2015 Nov 1;232(21–22):4085–97.
18. Bhattacharya S, Khatri A, Swanger SA, DiRaddo JO, Yi F, Hansen KB, et al. Triheteromeric GluN1/GluN2A/GluN2C NMDARs with Unique Single-Channel Properties Are the Dominant Receptor Population in Cerebellar Granule Cells. Neuron. 2018 Jul;99(2):315-328.e5.
19. Scherzer CR, Landwehrmeyer GB, Kerner JA, Standaert DG, Hollingsworth ZR, Daggett LP, et al. Cellular Distribution of NMDA Glutamate Receptor Subunit mRNAs in the Human Cerebellum. Neurobiol Dis. 1997 Jan 1;4(1):35–46.
20. Karavanova I, Vasudevan K, Cheng J, Buonanno A. Novel regional and developmental NMDA receptor expression patterns uncovered in NR2C subunit-β-galactosidase knock-in mice. Molecular and Cellular Neuroscience. 2007 Mar 1;34(3):468–80.
21. Monyer H, Burnashev N, Laurie DJ, Sakmann B, Seeburg PH. Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. Neuron. 1994 Mar;12(3):529–40.
22. Chatterton JE, Awobuluyi M, Premkumar LS, Takahashi H, Talantova M, Shin Y, et al. Excitatory glycine receptors containing the NR3 family of NMDA receptor subunits. Nature. 2002 Feb 30;415(6873):793–8.
23. Xi D, Keeler B, Zhang W, Houle JD, Gao WJ. NMDA receptor subunit expression in GABAergic interneurons in the prefrontal cortex: Application of laser microdissection technique. J Neurosci Methods. 2009 Jan 30;176(2):172–81.
24. Currie J, Joyce S, Maruff P, Ramsden B, McArthur-Jackson C, Malone V. Selective impairment of express saccade generation in patients with schizophrenia. Exp Brain Res. 1993;97(2).
25. Hever F, Sahin D, Aschenbrenner S, Bossert M, Herwig K, Wirtz G, et al. Visual N80 latency as a marker of neuropsychological performance in schizophrenia: Evidence for bottom-up cognitive models. Clinical Neurophysiology. 2021 Apr;132(4):872–85.
26. George DT, Ameli R, Koob GF. Periaqueductal Gray Sheds Light on Dark Areas of Psychopathology. Trends Neurosci. 2019 May;42(5):349–60.
27. Almada RC, Genewsky AJ, Heinz DE, Kaplick PM, Coimbra NC, Wotjak CT. Stimulation of the Nigrotectal Pathway at the Level of the Superior Colliculus Reduces Threat Recognition and Causes a Shift From Avoidance to Approach Behavior. Front Neural Circuits. 2018 May 7;12.
28. Liang F, Xiong XR, Zingg B, Ji X ying, Zhang LI, Tao HW. Sensory Cortical Control of a Visually Induced Arrest Behavior via Corticotectal Projections. Neuron. 2015 May;86(3):755–67.

ANNEX

Table 1

A. One-way ANOVA (Experiment 1)

	Treatment		
	F(DFn, DFd)	p value	
SC superficial	F (2,8)= 0,086	P > 0.05	ns
SC lateral	F (2,8)= 5,270	P > 0.05	ns
SC medial	F (2,8)= 2,135	P > 0.05	ns
PAG	F (2,8)= 1,065	P > 0.05	ns

B. Two-way ANOVA (Experiment 2)

	Treatment			Genotype			Interaction		
	F(DFn, DFd)	p value		F(DFn, DFd)	p value		F(DFn, DFd)	p value	
SC superficial	F (1, 15) = 0.076	P > 0.05	ns	F (1, 15) = 5.270	P < 0.05	*	F (1, 15) = 5.407	P < 0.05	*
SC lateral	F (1, 15) = 14.35	P < 0.05	*	F (1, 15) = 0.063	P > 0.05	ns	F (1, 15) = 0.075	P > 0.05	ns
SC medial	F (1, 15) = 3.151	P > 0.05	ns	F (1, 15) = 0.009	P > 0.05	ns	F (1, 15) = 0.009	P > 0.05	ns
PAG	F (1, 15) = 0.017	P > 0.05	ns	F (1, 15) = 2.436	P > 0.05	ns	F (1, 15) = 2.436	P > 0.05	ns

C. Two-way ANOVA (Experiment 3)

	Treatment			Genotype			Interaction		
	F(DFn, DFd)	p value		F(DFn, DFd)	p value		F(DFn, DFd)	p value	
SC superficial	F (2, 23) = 3.867	P < 0.05	ns	F (1, 23) = 0.385	P > 0.05	ns	F (2, 23) = 0.786	P > 0.05	ns
SC lateral	F (2, 23) = 17.45	P < 0.001	**	F (1, 23) = 1.553	P > 0.05	ns	F (2, 23) = 0.398	P > 0.05	ns
SC medial	F (2, 23) = 12.19	P < 0.001	**	F (1, 23) = 2.441	P > 0.05	ns	F (2, 23) = 0.729	P > 0.05	ns
PAG	F (2, 23) = 14.25	P < 0.001	**	F (1, 23) = 1.651	P > 0.05	ns	F (2, 23) = 0.294	P > 0.05	ns

D. Two-way ANOVA and Mann-Whitney test (Experiment 4)

Two-way ANOVA

♂

	Treatment			Genotype			Interaction		
	F(DFn, DFd)	p value		F(DFn, DFd)	p value		F(DFn, DFd)	p value	
SC lateral	F (1, 14) = 0.577	P > 0.05	ns	F (1, 14) = 1.540	P > 0.05	ns	F (1, 14) = 1.536	P > 0.05	ns
PAG	F (1, 14) = 1.392	P > 0.05	ns	F (1, 14) = 0.033	P > 0.05	ns	F (1, 14) = 0.019	P > 0.05	ns

♀

	Treatment			Genotype			Interaction		
	F(DFn, DFd)	p value		F(DFn, DFd)	p value		F(DFn, DFd)	p value	
SC lateral	F (1, 20) = 2.012	P > 0.05	ns	F (1, 20) = 0.197	P > 0.05	ns	F (1, 20) = 0.197	P > 0.05	ns
PAG	F (1, 20) = 1.248	P > 0.05	ns	F (1, 20) = 0.643	P > 0.05	ns	F (1, 20) = 0.729	P > 0.05	ns

Mann-Whitney test

♂

		p value	Mean rank of SAL	Mean rank of KET30	Mann-Whitney U	q value	
	WT	P > 0.05	4,500	4,500	8,000	P > 0.05	ns
SC superficial	**KO**	P > 0.05	7,400	3,600	3,000	P > 0.05	ns
	WT	P > 0.05	3,000	6,000	2,000	P > 0.05	ns
SC medial	**KO**	P > 0.05	6,800	4,200	6,000	P > 0.05	

♀

		p value	Mean rank of SAL	Mean rank of KET30	Mann-Whitney U	q value	
	WT	P > 0.05	5,500	7,500	12,00	P > 0.05	ns
SC superficial	**KO**	P > 0.05	5,500	7,500	12,00	P > 0.05	ns
	WT	P > 0.05	5,250	7,750	10,50	P > 0.05	ns
SC medial	**KO**	P > 0.05	5,167	7,833	10,00	P > 0.05	

Table 1. Summary of statistical analysis from experiment 1 to 4. Superficial Superior Colliculus (SC superficial), Lateral Superior Colliculus (SC lateral), Medial Superior Colliculus (SC medial), Periaqueductal gray matter (PAG). * P<0,05, **P<0,01, non-significant (ns).