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Optogenetic activation of granule cells in the dorsal dentate gyrus enhances dopaminergic neurotransmission in the Nucleus Accumbens

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Abstract

The dentate gyrus (DG) has distinct roles along its dorso-ventral axis. In the mouse, we recently demonstrated that dorsal DG(dDG) stimulation enhances exploratory behavior(Kheirbek et al., 2013). Dopamine (DA) release in the Nucleus Accumbens (NAcc), which belongs to the reward system, could be a key target of dDG mediating this motivation-related behavior. Here, an optogenetic stimulation of either ventral (vDG) or dDG granule cells was coupled with NAcc DA release monitoring using *in vivo* microdialysis. Only dDG stimulation enhanced NAcc DA release, indicating differential interconnections between dDG and vDG to the reward system.

Keywords

Optogenetics; Microdialysis; Hippocampus; Dopamine

The hippocampus (HPC) is classically subdivided into dorsal and ventral parts. The dorsal HPC (dHPC) is mainly associated with memory function, whereas the ventral HPC (vHPC) is associated with emotion and mood (Fanselow and Dong, 2010). Such a sub-division was also reported regarding differential pattern of gene expression (Leonardo et al., 2006). Circuit tracing studies showed also that the output of the HPC differs along the dorso-ventral axis, indicating that depending on the locus of hippocampal output, different downstream structures may be recruited (Fanselow and Dong, 2010). Studying the functional

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.neures.2017.12.002.

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consequences of this dorso-ventral subdivision of the hippocampal downstream pathways would help in a detailed understanding of the HPC function. For this purpose, optogenetics is a promising tool ((Yizhar et al., 2011) for a review). In this context, a recent study using optogenetics found that dorsal dentate gyrus (dDG) stimulation enhanced exploratory behavior, while activation of the ventral dentate gyrus (vDG) produced a decrease in anxiety-related behavior (Kheirbek et al., 2013). Different downstream brain regions related either to the dDG or vDG could explain these differential effects of the stimulation. Some evidences suggest that behavioural effects of vDG stimulation could be related to the amygdala (Bienvenu et al., 2012; Kishi et al., 2006; Tye et al., 2011) and/or the prefrontal cortex (Adhikari et al., 2010). By contrast, the dDG could control exploratory behavior through its projection from CA3 to the lateral septum and ventral tegmental area (VTA) (Luo et al., 2011; Swanson, 2000). Considering the involvement of these latter brain regions in the reward pathway, which is a major system enhancing motivational behavior (Salamone et al., 2016), we hypothesized that dDG stimulation could enhance dopamine (DA) release in the Nucleus Accumbens (NAcc), which is an important target of VTA dopaminergic neurons. Our hypothesis is also supported by the fact that the increase in exploration enhanced by the dDG stimulation was suppressed by a dopamine D1 receptor antagonist (SCH-23390) (Kheirbek et al., 2013).

In the present study, we used the previously described optogenetic protocol to stimulate granule cells either from the dDG or vDG (Kheirbek et al., 2013). We coupled these stimulations with *in vivo* microdialysis in freely moving mice in order to measure changes in extracellular levels of DA in the NAcc. It allowed us to validate our hypothesis, of the involvement of the DA release in the Nacc induced by the dDG stimulation.

To target opsin expression selectively to granule cells in the DG of the HPC, a mouse line expressing specifically Cre recombinase in this cell type has been chosen: the proopiomelanocortin (POMC)–bacterial artificial chromosome (BAC) Cre recombinase line (McHugh et al., 2007). POMC is strongly expressed in all the layers of the DG and is selective of granular cells (as defined by immunohistochemistry for $NeuN^+$, $S100b^-$ and $GAD\ 67^-$) (McHugh et al., 2007). This line was crossed to conditional mouse lines that contained the blue light-activated cation channel channelrhodopsin (ChR2) (Boyden et al., 2005) (ROSA26-CAG-stopflox-ChR2(H134R)- YFP (Madisen et al., 2012). These mice, referred as POMC-ChR2 mice, were compared to wild type animals (without ChR2 expression) from the littermate, used as control. Adult male (n=14) and female (n=13) mice equally distributed in all groups of the C57/BL6/129 sv background (10–15 weeks-old) were used. They were housed in groups of 4–6 animals per cage and under an alternating light–dark cycle (lights on from 7:00 to 19:00 h) at 21+/−1 °C. All the experiments have been performed during the light period between 11 AM and 3 PM. All testing were conducted in compliance with the laboratory animal care guidelines, with protocols approved by the Institutional Animal Care and Use Committee (CEE26 authorization 2013–091, permissions # 92–196" to A.M.G.) as well as with the European directive 2010/63/EU.

For all experiments, a 200 μm core, 0.37 numerical aperture (NA) multimode fiber (ThorLabs) was used for optical stimulation via a patch cable connected to a 100 mw 473 nm laser diode (OEM laser systems) (Sparta et al., 2011). Mice were anaesthetized (chloral

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hydrate 400 mg/kg i.p.) and surgically implanted unilaterally with a fiber optic targeted to the DG either in the dorsal part [stereotaxic coordinates (from Bregma): −1.4mm AP, −1 mm LL, −1.75 mm DV] or in the ventral part (−3.4 mm AP, −2.25 mm LL, −2.25 mm DV) according to (Franklin and Paxinos, 2007). Mice were also implanted with concentric microdialysis probes made of cuprophan fibers (active length 2 mm, outer diameter 0.3 mm) located in the NAcc (1.4 mm AP, ±0.7 mm LL, −4.5 mm DV) as already described (Maskos et al., 2005). Animals were allowed to recover from the surgery overnight. The next day, the probes were continuously perfused with aCSF (composition in mM: NaCl 147, KCl 3.5, $CaCl_2$ 1.26, $MgCl_2$ 1.2, $NaHCO_3$ 25, NaH_2PO_4 1.0, pH 7.4 ± 0.2) at a flow rate of 1.0 $\mu L min^{-1}$ using a CMA/100 pump (Carnegie Medicin, Stockholm, Sweden) as previously described (Gardier et al., 2003). The animals were awake and freely moving in their cage during the microdialysis/optogenetic protocol. Dialysates were collected every 25min for 100 min before (basal conditions B0 at T0) and for another 100 min after the optogenetic stimulation (post-illumination period). At T100, mice received blue light application *via* the fiber optic during 5 min with 5 ms pulses with a frequency of 10 Hz and a power of 8–10 mW. These parameters have been previously determined in the study of (Kheirbek et al., 2013) using the same strain of mice and demonstrating the link between dDG stimulation and the exploratory drive.

Samples were analyzed for DA levels using HPLC (column Ultremex 3 μ C18, 75 × 4.60 mm, particle size 3 μm, Phenomenex, Torrance, CA) coupled to an amperometric detector (VT03; Antec Leyden, The Netherlands). The limit of sensitivity for DA concentration was 0.5 fmol per sample (signal-to-noise ratio = 2). Basal values were averaged and used as a baseline (B_0) to calculate percent changes following the optogenetic stimulation.

For confirmation of probe placement, mice were cervically dis-located, the brains were quickly removed, frozen on dry ice and stored at −80 °C until sectioning into 20 μm coronal slices mounted directly onto slides. Sections were fixed for one hour in 4% PFA, incubated for 1 min in cresyl violet (0.3%), dehydrated in ethanol, and coverslipped with Eukitt (Sigma Aldrich, St Louis, MO). Images of the sections were scanned (Epson perfection V500) and inserted in Adobe Illustrator®, and probe location was determined (Supplementary Fig. 1). Animals presenting inadequate probes location were removed. Typical location of the optode dedicated to the stimulation of either the dDG or the vDG are presented respectively in Supplementary Fig. 1b and c. In these cases, the optodes are located on the top of the dentate gyrus allowing an illumination of underlying brain regions (Yizhar et al., 2011). Typical location of the microdialysis probes in the NAcc is presented in the Supplementary Fig. 1d. The active part of the membrane consists in the 2 mm located up to the tip of the probe (see Supplementary Fig. 1e and f).

Data are expressed as mean ± standard error of the mean (s.e.m.). Statistical analyses were processed with Prism 6.0 software (GraphPad). Data were analyzed by two-way ANOVA. Significant main effects and interactions were followed by Bonferroni post-hoc analyses. Differences were considered significant when $p \leq 0.05$.

We used *in vivo* microdialysis in freely moving mice in order to measure changes in extracellular levels of DA (DA_{ext}) in the NAcc following an optogenetic stimulation either of

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the dDG (Fig. 1A) or the vDG (Fig. 1B). In WT, the B_0 used as reference were: 20.1 ± 5,1 fmol/sample; 20 ± 5.7 fmol/sample for dorsal and ventral stimulation, respectively. In POMC-ChR2 animals, the B_0 used as reference were: 11 ± 2.0 fmol/sample; 12.5 ± 5.6 fmol/sample for dorsal and ventral stimulation, respectively. Concerning the dDG stimulation, two-way ANOVA (genotype × time) of DA_{ext} revealed significant effects of genotype ($F_{1,88} = 10.43$, $p = 0.0017$), time ($F_{7,88} = 17.11$, $p < 0.001$) and a significant genotype × time interaction ($F_{7,88} = 18.18$, $p < 0.001$). The Bonferroni *post-hoc* analysis revealed that the increase in DA_{ext} was restricted to the dialysate samples corresponding to the optogenetic illumination period (i.e., when dialysates were collected at the time 125 min), in POMC-ChR2 mice ($p < 0.001$). This immediate and transient increase in DA_{ext}, in POMC-ChR2 mice was observed after the dDG stimulation returned to baseline in the next dialysate (collected at the time 150 min, Fig. 1A). Concerning the vDG stimulation, two-way ANOVA (genotype × time) of DA_{ext} revealed no effects of genotype ($F_{1,94} = 1.22$, $p = 0.27$), no effect of time ($F_{7,94} = 1.27$, $p = 0.28$) and no genotype × time interaction ($F_{7,94} = 0.57$, $p = 0.78$). Altogether, these data suggest a selective induction of DA release in the NAcc by an optogenetic illumination on dDG.

In the present study, we successfully measured NAcc DA release in transgenic POMC-ChR2 mice selectively expressing ChR2 in the granule cells of the DG in the HPC. Our findings indicate, with our stimulation parameters, that the dDG activity, but not vDG, controls DA release in the NAcc. These observations are in accordance with another optogenetic study demonstrating that ChR2 stimulation of the VTA was able to reproduce natural patterns of DA release in rat striatum (Bass et al., 2010). The exploratory behavior induced by a dDG optogenetic stimulation was abolished by a dopaminergic receptor antagonist (Kheirbek et al., 2013). The present results highlight a crucial role of the dDG-induced DA release in the exploratory behavior. However, dHPC and vHPC (more specifically the CA3) are both connected to the VTA (Lisman and Grace, 2005). A study using a pseudo-rabies virus, as retrograde trans-synaptic tracer, confirmed the connections between the CA3 region of the HPC and the VTA through the caudo-dorsal Lateral Septum (cdLS) (Luo et al., 2011). Interestingly, in the same study, the cdLS lesion, affected dorsal, but not ventral CA3 labeling (Luo et al., 2011). It shows that CA3-cdLS-VTA projections mainly involved dHPC projections. Finally, if the VTA is targeted by both dDG and vDG of the HPC, the anatomical projections of the VTA could be differential and may depend on the origin of the stimulation (in our case dDG or vDG). For example, a selective neuronal loop does exist between vHPC, VTA and HPC itself; inducing DA release in the HPC (Lisman and Grace, 2005). Altogether, it makes us to consider that the optogenetic dDG stimulation can induce DA release in the Nacc via the CA3- cdLS- VTA pathway (Fig. 2).

The link between the HPC and Nacc DA release is rather complex. Several data obtained in rodents showed that the HPC formation projects glutamatergic fibers from the hippocampal formation directly in the Nacc (Groenewegen et al., 1987). Such projections target NMDA receptors located on GABAergic interneurons controlling dopaminergic terminals (Boulenguez et al., 1998; French and Totterdell, 2003; van Dongen et al., 2005). The NMDA receptor stimulation in either dHPC or vHPC induced then differential consequences on extracellular DA levels in the Nacc (Peleg-Raibstein and Feldon, 2006). In this study, NMDA infusion into the dHPC decreased DA release in the Nacc, whereas an increase was

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observed in the Nacc following vHPC stimulation (Peleg-Raibstein and Feldon, 2006). The same vHPC-Nacc link was highlighted with the use of a 5-HT_{1B} receptor agonist, demonstrating an inhibitory effect of serotonin system in this pathway (Boulenguez et al., 1996). The discrepancy between these studies and our results is likely due either to differences in stimulation parameters, or to differential pathway recruitment. First, with the optogenetic stimulation here, the granule cells of the DG have been targeted selectively, which is not the case with intra-hippocampal NMDA application. Second, the 10 Hz stimulation, we used, is a frequency near to the theta frequency. Such a theta frequency applied in the dorsal CA3 region induced a 3-fold increase in the spikes frequency of VTA dopaminergic neurons (Luo et al., 2011). Such a strong increase in the electrical activity is certainly stronger than that induced by the hippocampal-Nacc direct pathway activation, thus leading to an inhibition of the DA release (Peleg-Raibstein and Feldon, 2006). Furthermore, the electrical activity increase following the theta stimulation is in the same range that the one we observed here in DA release in the Nacc suggesting that the pathway linking the dDG to the Nacc in our experimental conditions of stimulation implicated the VTA (Fig. 2). Even if optogenetics could induce non-physiological stimulation (massive and instantaneous), this experiment is a circuit mapping experiment, which identifies a pathway that leaves the dDG, but not vDG, in the control of reward circuits (whose activation induces an increase in DA content in the Nacc dialysates; see Supplementary Fig. 1g).

Altogether, our results showed a functional subdivision between dDG and vDG where optogenetic stimulation of the dDG was able to enhance DA release in the Nacc. It demonstrates that the link between the dHPC and the VTA (Luo et al., 2011) is functional. These differential interconnections between dDG and vDG to the reward system could explain the exploratory behavior observed following the same experimental protocol of dDG stimulation (Kheirbek et al., 2013).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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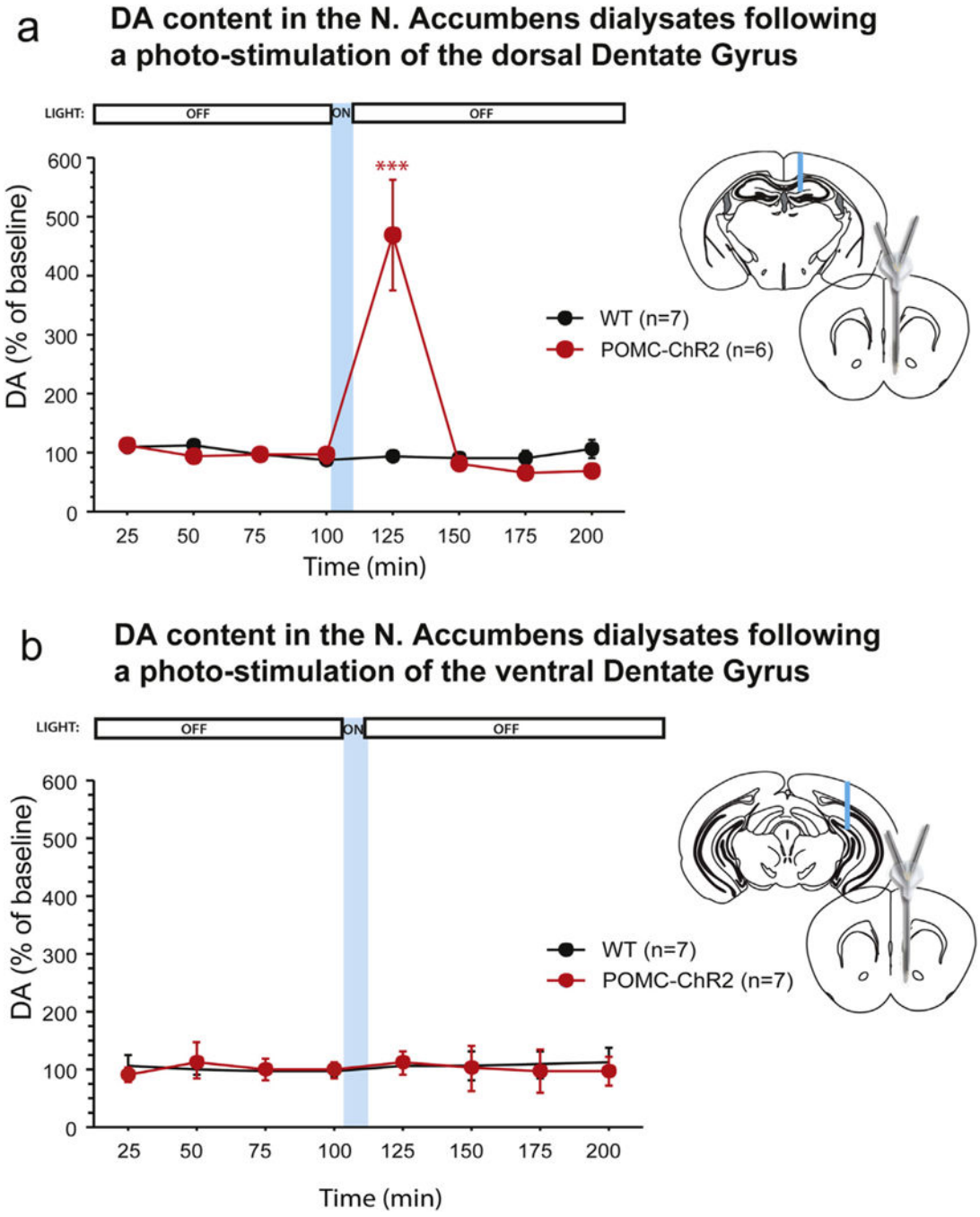

Fig. 1.

Optogenetic stimulation of the dorsal Dentate Gyrus induces DA release in the Nucleus Accumbens in mice, whereas the same stimulation of the ventral Dentate Gyrus was inefficient.

Time course of changes in extracellular dopamine (DA_{ext}) levels, shown as percentage of baseline, in the NAcc in POMC-ChR2 (blue) and WT mice (black). Mice received blue light application (5 ms pulses, 10 Hz for 5 min, schematically represented by the blue bar) to the dorsal Dentate Gyrus (A) or to ventral Dentate Gyrus (B). The data are presented as means ±

S.E.M., n = 6 to 7 animals per group. Statistical significance refers to POMC-ChR2 vs WT comparisons: ***p < 0.001

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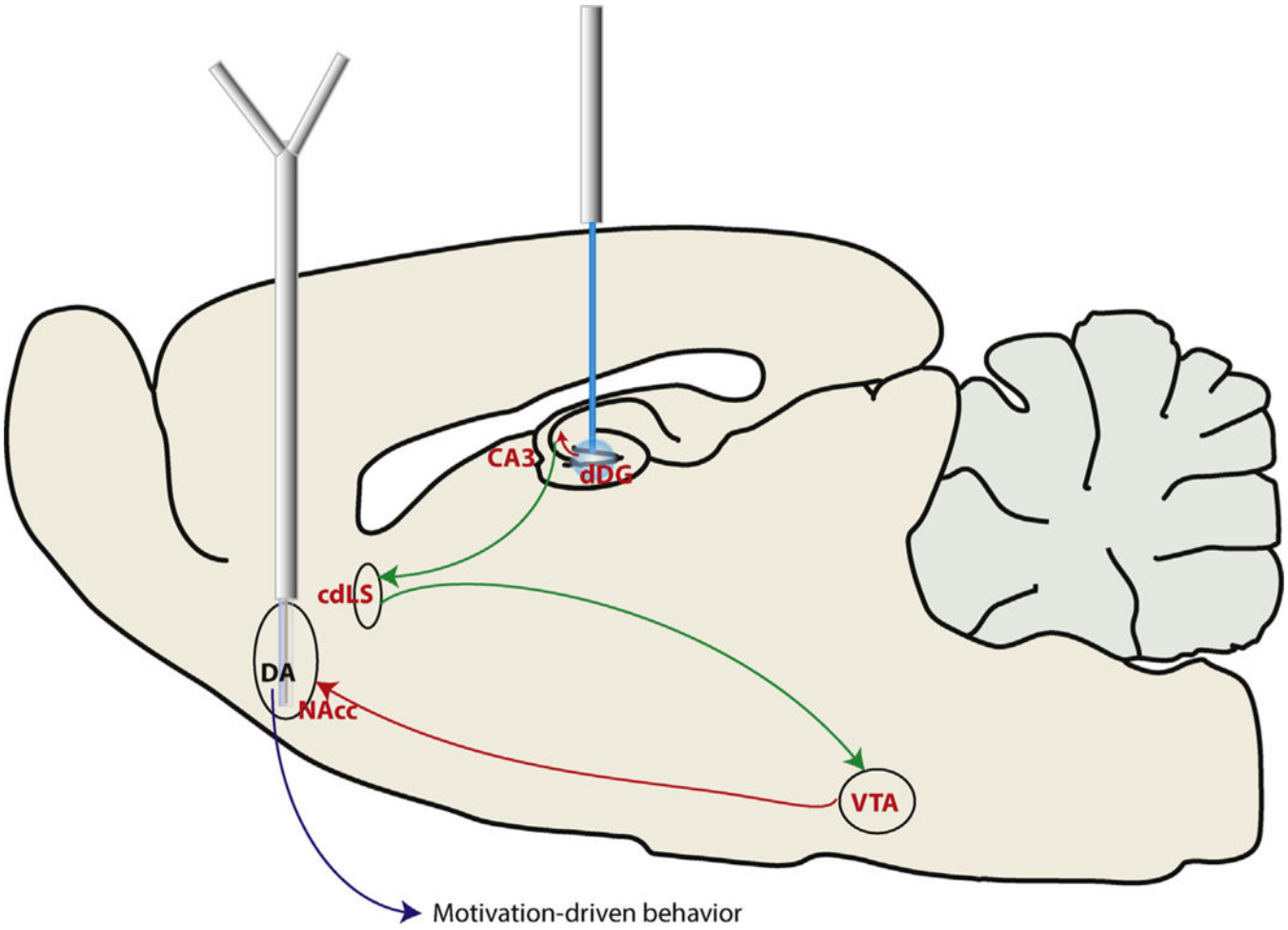

Fig. 2.

Pathway affected by the Optogenetic stimulation of the dorsal Dentate Gyrus.

The activated granule cells project to the CA3 through the mossy fibers. The CA3 pyramidal cells projects then to the caudo-dorsal Lateral Septum (cdLS), which is linked to the Ventral Tegmental Area (VTA): this pathway (green arrows) was highlighted by Luo et al. (2011). The present work showed that in response to the Optogenetic stimulation, dopaminergic neurons from the VTA released DA in the Nucleus Accumbens (NAcc), where a microdialysis probe was implanted (red arrows). Such a dopamine release is certainly involved in the exploratory behavior described by Kheirbek et al. (2013) (blue arrow) using the same experimental model as ours.