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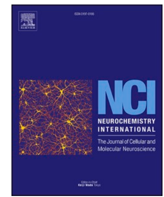

There's no place like home? Return to the home cage triggers dopamine release in the mouse nucleus accumbens

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ABSTRACT

Various stimuli have been employed as reinforcers in preclinical rodent models to elucidate the underpinnings of reward at a molecular and circuit level, with the release of dopamine (DA) in the nucleus accumbens (NAc) as a well-replicated, physiological correlate. Many factors, however, including strain differences, sex, prior stress, and reinforcer administration protocols can influence reward responding and DA release. Although previous evidence indicates that access to the home cage can be an effective reinforcer in behavioral tasks, whether this simple environmental manipulation can trigger DA release in the NAc has not been demonstrated. Here, using fiber photometric recordings of *in vivo* NAc dopamine release from a genetically-encoded DA sensor, we show that the movement of animals from the home cage to a clear, polycarbonate recording chamber evokes little to no DA release following initial exposure whereas returning animals from the recording chamber to a clean, home-like cage or to the home cage robustly triggers the release of DA, comparable in size to that observed with a 10 mg/kg i.p. Cocaine injection in the recording chamber. Although DA release can be evoked in moving mice to a clean cage, this release was significantly augmented when moving animals from the clean cage to the home cage. Our data provide direct evidence that home cage return from a foreign environment results in a biochemical change consistent with that of a rewarding stimulus. This simple environmental manipulation provides a minimally invasive approach to study the reward circuitry underlying an ethologically relevant reinforcer, return to the safe confines of "home". The home cage – DA release paradigm may also represent a biomarker-driven paradigm for the evaluation of genetic and experiential events that underlie anhedonic states, characteristic of major mood disorders, and to present new opportunities to identify their treatments.

1. Introduction

The neurotransmitter dopamine (DA) is critical to motivational control and to directing behaviors that seek reward and have preferred outcomes (Wise 2004). DA release in the nucleus accumbens (NAc) of humans (Abler et al., 2006) and rodent models (Hernandez and Hoebel 1988) is triggered by reward and reward-coupled cues that underlie reinforcement learning, and is a convergent response identified with the rewarding properties of natural and pharmacological reinforcers. DA neurons operate as "reward prediction error" indicators, responding to both unexpected larger rewards, and to cues that signal an increase in future reward, with increased DA release (Bayer and Glimcher 2005). Recent findings broaden the types of stimuli that elevate DA neuronal activity beyond those strictly defined as rewarding, to include unexpected, novel, salient, and even aversive stimuli (de Jong et al., 2019; Horvitz 2000; Lammel et al. 2014).

Several studies indicate that the home cage environment is a rewarding stimulus for rodents. At the beginning of 20th century, Rietta Simmons assessed various rewards for their potential to motivate maze acquisition and observed that "home cage return" was a weak reinforcer in that regard (Simmons 1924). More recently, Blizzard and colleagues demonstrated that mice allowed immediate access to their home cage after reaching the goal box of a Lashley III maze learned the acquisition of the maze at a comparable rate to food-deprived mice that received food as a reward (Blizard et al. 2003, 2006). As described above, DA neuron activity and DA release is sensitive to stimuli that signal valence

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and saliency for motivational learning that include reward and novelty. To our knowledge, however, returning to the home cage has yet to be explored for its connection to DA release.

With the advent of techniques that allow for recordings of distinct neuronal populations in freely behaving animals with high sensitivity and time resolution, responses to minimally-stressful procedures such as change in cage environment are feasible. In this study we used fiber photometry to monitor DA release in the NAc of mice stereotaxically-injected with a genetically encoded, G-protein coupled receptor-activation based dopamine ($GRAB_{DA}$) sensor (Sun et al., 2018) in response to relocation from the home cage to a novel arena (hereafter noted as a recording chamber), and when subsequently returned to the home cage or a clean, unused cage with home cage-type bedding. We hypothesized that mesolimbic DA projections from the ventral tegmental area to the ventral striatum, including the NAc, are involved in the rewarding nature (Russo and Nestler 2013; Salamone et al., 2005) of home cage return. Indeed, our results demonstrate that unlike transfer from the home cage to a recording chamber, return to the home cage or a clean cage significantly elevates DA release that is temporally aligned with transfer. Whereas movement from the home cage to a cage with clean bedding evoked DA release, this release was quantifiably less than that observed when animals were transferred from the clean cage back to the home cage. These responses support the ability of environmental changes to drive NAc DA release and suggest that the use of this paradigm may allow for a characterization of circuits that support the response to safe or rewarding environments and that align with, or are distinct from, the response to other reinforces such as drugs of abuse. They also support the use of this simple model to explore anhedonic states produced by gene mutations or environmental stressors.

2. Materials and methods

2.1. Animals

All experiments described were performed with 9–10 week-old male mice of a hybrid background (~75% 129S6/SvEvTac and ~25% C57BL/6J). Mice were housed in 13 inch long x 7 inch wide x 5.7 inch deep polycarbonate cages (Super Mouse 750™ ventilated cage, Lab Products Inc., Seaford, DE, USA) on a reverse light cycle (light on/off at 3 p.m./3 a.m., respectively) until 5 weeks of age, at which time they were moved to a standard light cycle (light on/off at 7 a.m./7 p.m., respectively) prior to surgeries and fiber photometry studies as described below. Food and water were provided *ad libitum*. All studies were performed under a protocol approved by the Institutional Animal Care and Use Committee (IACUC) at Florida Atlantic University.

2.2. Materials

To monitor *in vivo* DA release, we utilized a genetically-encoded, G-protein coupled receptor -activation-based DA sensor ($GRAB_{DA}$) (Sun et al., 2018), delivered via an adenovirus associated viral (AAV) vector AAV-hSyn-DA2m (serotype 9) (Vigene Biosciences, Inc., Rockville, MD USA) (Sun et al., 2021). As a control, we utilized a DA-insensitive tdTomato reporter expressed by AAV9-hSyn-tdTomato (serotype 9, Vigene Biosciences, Inc., Rockville, MD USA). Cocaine hydrochloride was purchased from Sigma Aldrich (St. Louis, MO, USA). A 15-inch-high clear animal enclosure with holes for water and feeding (MTANK W/F (Instech, Plymouth Meeting, PA, USA)), commonly used for microdialysis, served as the standard recording chamber for all experiments.

2.3. Viral injections and optical fiber implantation

6-week old male mice were anaesthetized (isoflurane; 5% induction and 2% maintenance) and immobilized in a stereotaxic frame (Kopf Instruments, Tujunga, CA, USA) with ophthalmic ointment applied. Ketoprofen (10 mg/kg, subcutaneous injections) and bupivacaine (1–5 mg/kg/0.1 ml/local subcutaneous injections)/lidocaine (2–5 mg/kg/0.1 ml/local subcutaneous injection) where administered under aseptic conditions following guidelines approved by the IACUC at Florida Atlantic University. Following a midline incision, lambda and bregma were leveled, and two 1.6 mm screws (Plastics One Inc., 00-96X1/16 39052; purchased from Fisher Scientific) were affixed to the skull and a dental drill was used for the craniotomy.

The NAc was targeted using the following coordinates: anterior/posterior: +1.54 mm; medial/lateral: 0.6 mm; dorsal/ventral: -4.1 mm; all relative to bregma). A 34-gauge metal needle attached to 10 μL Nanofil Hamilton syringe (Hamilton, Reno, NV, USA) was carefully lowered into the NAc using the coordinates given above, with the exception that the tip of the needle was positioned at dorsal/ventral −4.125 mm, and was held in place for 2 min and slowly withdrawn to dorsal/ventral −4.1 and then held in place for an additional 5 min prior to the injection of the AAVs. A final volume of 1 μL containing AAV-hSyn-DA2m and AAV9-hSyn-tdTomato (1.0×10^{10} and 0.6×10^{10} genome copies, respectively) was injected at an infusion rate of 100 nL per minute, with the needle subsequently kept at the injection site for 10 min and then slowly withdrawn. Subsequently, an optical fiber was lowered into the NAc (anterior/posterior: +1.54 mm; medial/lateral: 0.6 mm; dorsal/ventral: -4.1 mm; all relative to bregma) and secured to the skull with dental acrylic. Surgeries for viral injection and fiber implantation occurred at least 21 days prior to the recordings to allow for robust reporter expression. Animals were singly-housed after surgery. The mice spent a minimum of 48 h in the cage that was defined as "home cage". Mice were not habituated to the recording chamber. A schematic representation of the probe placement and viral spread are shown in the Supplementary Fig. 1.

2.4. In vivo fiber photometry to detect DA release

To monitor the signal from both the $GRAB_{DA}$ sensor and the DA-insensitive tdTomato reporter, two light-emitting diodes (465 and 560 nm; CLED_465/CLED_560, Doric Lenses, Quebec, QC, Canada), reflected through dichroic mirrors were coupled to a 200 μm core/225 μm cladding diameter optical fiber (Thorlabs, Newton, NJ, FP200URT, High OH multimode fiber, 0.5 numeric aperture) glued to a metal ferrule (Doric Lenses, Quebec, QC, Canada), which was implanted into the NAc as noted above. Emitted light was band-pass filtered (460–490 nm; 580–680 nm, FMC6, Doric Lenses, Quebec, QC, Canada) and detected by a Newport Femtowatt silicone PIN photodetector (New Focus, San Jose, CA, USA). Fiber photometry data were acquired with Synapse Software controlling an RZ5P lock-in amplifier (Tucker-Davis Technologies, Alachua, FL, USA). 465 nm and 560 nm sinusoidal excitation was delivered at 210Hz and 450Hz, respectively, by an LED driver (Doric lenses, LEDD_4) at low power mode. A demodulated signal was low-pass filtered at 6 Hz and digitized at 1017 Hz. Data were analyzed with OriginPro (OriginLab, Northampton, MA, USA) and processed with Microsoft Excel (Microsoft, Redmond, WA, USA) and Prism 8 (GraphPad, San Diego, CA, USA). A USB camera (Logitech webcam, 1080p) was used to simultaneously record animal behavior and photometry signals.

To monitor dopamine release upon transfer to either the home cage, a clean cage, or a recording chamber, we used five individual mice (Fig. 3 A and B). Each mouse was placed into 15-inch-high clear recording chamber with holes for water and feeding (MTANK W/F (Instech, Plymouth Meeting, PA, USA)) without bedding. After the signal stabilized (typically after 200 s), the mouse was carefully transferred into either its home cage (i.e. a cage in which the mouse spent a minimum of 48 h with nestlet and bedding) or a clean cage (i.e. a clean cage identical to the home cage with fresh bedding and an untouched nestlet). After 100 s, the mouse was carefully transferred back into the recording chamber for another 100 s before the mouse was transferred into either its home cage or a clean cage. This procedure was performed in a randomized fashion. To monitor dopamine release upon transfer from the home cage to a clean cage or *vice versa*, three mice were transferred from

the home cage to a clean cage and *vice versa.* For cocaine injections (10 mg/kg i.p.), mice were removed from the home cage, carefully injected and placed directly into the recording chamber where they remained throughout the recording session. Cocaine was injected last in the recording chamber.

2.5. Immunohistochemistry

Sites of injection were validated by immunostaining of virally-encoded transgenes. After sacrifice, brains were removed and drop-fixed in 4% paraformaldehyde overnight and then stored in 30% sucrose in phosphate-buffered saline (PBS – 137 mM NaCl, 1.47 mM KH_2PO_4, 8.10 mM Na_2HPO_4, pH 7.3) until sectioning. Brains were imbedded in agarose and 40μm-thick NAc-containing brain sections were collected using a vibrating microtome (Precisionary Compresstome VF-300-0Z). Slices were stored in 0.02% sodium azide in PBS until staining. Slices were blocked in 2.5% normal donkey serum/2.5% normal goat serum in PBS +0.2% Triton X-100 (Sigma Aldrich Triton X-100 9002-93-1) for 1 h at room temperature, and then incubated in chicken anti-green fluorescent protein (GFP) antibody (Abcam ab13970) at a dilution of 1:500 and rabbit anti-red fluorescent protein (RFP) antibody at a dilution of 1:1000 (Abcam ab 124754) overnight at 4oC. Slices were then incubated in anti-rabbit Alexa Fluor 647 (Invitrogen A31573) and anti-chicken Alexa Fluor 488 (Invitrogen A11039) secondary antibodies for 2 h at room temperature. Between steps, sections were washed 3 times in PBS for 5 min each. Sections were mounted to slides and once dried were put through dehydrating and clearing steps - 70% ethanol, 90% ethanol, 100% ethanol, CitriSolv, CitriSolv (Decon Labs 1601) - for 5 min each step. Coverslips were attached using DPX mountant (Sigma-Aldrich 06522) which contains xylene for further clearing. Slices were imaged using a Nikon A1R confocal microscope at 2× and 20× magnification.

2.6. Data analyses

To quantify the DA signal arising from cocaine injections, we calculated the ratio of the DA-sensitive signal at 465 nm over the DA-insensitive signal at 560 nm, and quantified the difference between signals at $t = 50.125$ to $t = 149.9$ s immediately after the drug injections to the average signal at $t = 950$ to $t = 1050$ s after injection, identified as the peak response post-hoc. To compare the DA signal in response to the exposure to either the home cage, clean cage or the recording chamber, we used the video footage to specify the time when the mouse touched the floor/bedding of each environment as $t = 10$ s. To quantify the DA signal in the different environments, we calculated the ratio of the DA-sensitive signal at 465 nm over the DA-insensitive signal at 560 nm which was normalized to basal signal at $t = 0$sec–8.68sec. Based on post-hoc evaluation of recording patterns, we defined the average between $t = 11.3$sec–13.5sec as peak. The average of the 465nm/560 nm ratio from $t = 0$sec to $t = 8.68$sec was set as baseline and subtracted from the individual peaks. Shapiro-Wilk's test was used to confirm normal distribution of the data and Bartlett's plus Brown-Forsythe's test were used to test for significant differences in the standard deviations of the data sets. Data shown in Fig. 3B were analyzed using one-way ANOVA followed by Dunnett's multiple comparison test. Data shown in Fig. 3D were analyzed with a one-tailed, paired Student's t-test based on the hypothesis that the home cage would show an increased DA response compared with a clean cage that had not been lived in.

3. Results

To verify that the $GRAB_{DA}$ sensor reliably reports elevations in extracellular DA as previously described (Sun et al., 2018), we injected mice with cocaine hydrochloride (10 mg/kg, i.p.). As expected, owing to its antagonistic activity at the DA transporter (Kristensen et al., 2011) and its effect on the reward circuitry (Russo and Nestler 2013), we observed a significant increase in the DA sensitive signal (465 nm) relative to the DA-insensitive signal at 560 nm in the NAc (Fig. 1 A and B). The time course of the example trace (Fig. 1A) is in agreement with previous studies examining the plasma half-life of cocaine and the molecule's brain penetration, which is expected to peak at this dose around 15–20 min post-injection (Benuck Myron and Reith 1987; Wise et al. 2008).

To investigate whether return to a home cage environment elicits an increase in extracellular DA in the NAc, and encouraged by previous studies that demonstrate home cage return as a positive reinforcer (Taniuchi et al. 2019; Blizard et al. 2003, 2006; Bressler et al. 2010), we transferred an initial test mouse repeatedly between the standard recording chamber and the home cage in which the animal has been housed for more than 48 h. In these efforts, our recordings revealed a marked increase in extracellular DA in the NAc when the mouse was transferred from the recording chamber to the home cage. Fig. 2A depicts a representative time course of the raw signal of repeated translocations from the home cage to the recording chamber and *vice versa*, with the ratio of 465nm/560 nm signals shown in Fig. 2B. We observed that each translocation to the home cage from the recording chamber caused a marked increase in extracellular DA. Individual traces are shown at a higher temporal resolution in Fig. 2C–D and G-H). In contrast, translocation of the mouse to the recording chamber from the home cage resulted only in a blunted or no increase in the signal that typically occurred with all movements, followed by a return to pre-move baseline (Fig. 2A–B, E-F and I-J).

These observations were then extended to quantify the robustness of the home cage return effect on NAc DA release in a group of five male mice. Representative video recordings of animal transfers time-synched with fiber photometry traces are provided as Supplementary Movies 1, 2 and 3. In line with our initial recordings shown in Fig. 2, transfer of mice to the home cage caused a visibly evident elevation of extracellular DA (Fig. 3A, black trace). Since one of the original reports that demonstrated the reinforcing effect of returning to the home cage used a "pseudo-home cage that is the same size as the animal's standard home cage" (Bressler et al. 2010), which presumably refers to a cage that is identical to the cage in which the animal was housed, yet clean and with fresh bedding, we introduced this alternative destination into our protocol. In agreement with the reported reinforcing nature of return to a home-cage like environment (Bressler et al. 2010), transfer to the clean cage also triggered an increase in extracellular DA (Fig. 3A, green trace), comparable to the DA signals elicited by return to the home cage (Fig. 3A, black trace). Movement of mice from the home cage to the recording chamber (Fig. 3A, blue trace) did not trigger an increase in peak DA above pre-movement baseline in contrast to what was observed in movements from the recording chamber to either the clean cage or the home cage. Analysis of the initial peak in extracellular DA (at 11.3–13.5 s in Fig. 3A) with one-way ANOVA ($P = 0.0183$; Bartlett's test and Brown-Forsythe test with P greater than 0.05; Shapiro-Wilk test P greater than 0.05; $n = 5$ per group) followed by Dunnett's multiple comparison test revealed that the DA peak in response to a transfer to the home cage or to a clean cage differed significantly from the level of extracellular DA observed upon transfer from the home cage to the recording chamber (Fig. 3B). We further evaluated whether return to the home cage from a clean cage caused a higher increase in extracellular DA than transfer from the home cage into a clean cage in three additional male mice. Transfer from the home cage to the clean cage produced a transient elevation in extracellular DA. This effect was significantly smaller than when animals were transferred back to the home cage (one-tailed, paired Student's t-test, $P = 0.0008$; $n = 3$) (Fig. 3C and D).

Supplementary video related to this article can be found at https://doi.org/10.1016/j.neuint.2020.104894.

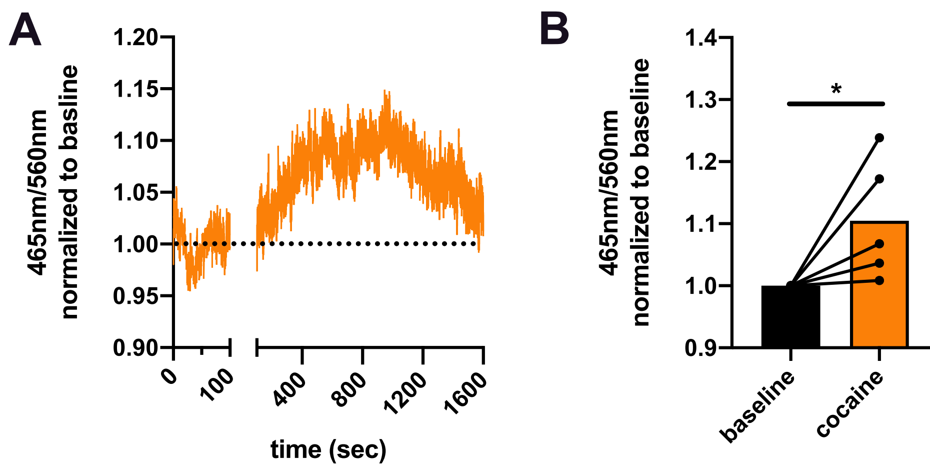

Fig. 1. DA release in the NAc following a single cocaine injection. (A) Shown is the ratio of the DA-sensitive signal (465 nm) over the DA-insensitive signal (560 nm) of one representative experiment, normalized to the average of the 465nm/560 nm ratio during the first 100 s. Cocaine (10 mg/kg, i.p.) was injected 50 s prior to the recording. (B) Displays the 465nm/560 nm ratio during the first 100 s (baseline) and from t = 950–1050 s (cocaine) divided by the average 465nm/560 nm ratio from t = 50.125 to t = 149.9. One-tailed Wilcoxon matched-pairs signed rank test; * = $P < 0.05$, n = 5.

4. Discussion

Our findings reveal immediate and robust elevations in extracellular DA in the NAc when mice change environments, specifically when they are returned to their home cage or a home cage-like environment (i.e. a clean cage) from a standard recording chamber. Although, as expected, temporal differences are evident, the peak DA elevations observed with home cage return approached those obtained with a 10 mg/kg i.p. dose of cocaine (compare Figs. 1B and 3B). As little to no elevations in DA signals were observed in moving from the home cage to the recording chamber, relocation to a new environment *per se* does not explain our observations. Rather our findings are consistent with prior reports of access to the home cage upon completion of a behavioral task as reinforcing (Taniuchi et al. 2019; Blizard et al. 2003, 2006). Interestingly, as can be seen in our initial observations shown in Fig. 2C and D, the amplitude of the peak tends to decrease during the course of the experiment. This might be the attributed to handling-induced stress (Ghosal et al., 2015), since acute stress affects DA release in a biphasic manner (Puglisi-Allegra et al., 1991). Another potential cause for the observed trend could be that the rewarding stimulus devaluates upon repeated exposure. The baseline DA levels also trend to decrease over repeated transfers, possibly a result of diminished DA availability, though this interpretation requires more direct and repeated studies in more animals. Nonetheless, it may be important to limit the number of trials performed during one experimental session to reduce experimental variability. We observed that, in some traces, exposure to the recording chamber resulted in a slight increase in the DA signal (Fig. 2I and J). It is plausible that in these cases, the rise in DA signal could reflect i) interest in the novel environment (Rebec et al., 1996), ii) a mesolimbic relief signal, (Mayer et al., 2018; Navratilova et al. 2015), arising from the termination of the hold on their tail or iii) an increase in DA in response to an aversive stimulus. However, our probe placement and the time course of the signal do not match previous reports demonstrating the release of DA in response to aversive stimuli (de Jong et al., 2019).

When we compared the amplitude of the DA peak in response to transfers between the home cage, the recording chamber and a clean cage (Fig. 3B) we found that the responses to the home and clean cages were comparable. These results support the idea that the clean cage is recognized by mice as very similar to the home cage, and may reflect that the clean cage contains similar salient cues for the mice (familiarity, size, shape, bedding material) that signal home cage rewarding outcomes including food, water, nestlets, and social interaction or simply that movement to a clean environment cage so long as it looks like home is itself reinforcing. Regardless, this finding is consistent with a report of the rewarding nature of either a home cage or a home cage-like environment (Blizard et al. 2003, 2006). In contrast to the clean cage, the recording chamber bears little resemblance to the home cage, having different sides and floor materials, size and shape, and lack of bedding. We conclude that the recording chamber, as an environment presenting no recognizable rewards or cues indicating future reward, eliciting at best small DA responses related initially to novelty. Transfer from the home cage to a clean cage resulted in DA changes that were quantifiably lower than increases in DA observed upon transfer from the clean cage to the home cage (Fig. 3D) in the same subjects. This observation may indicate that cues associated with “home” (for instance test-subject derived odors or broken up nestlet) encode higher reward than the clean cage. Given at present the lower number of test-subjects in these studies (n = 3), this interesting finding should be evaluated in more detail in future studies. Finally, we observed that the overall DA signal and the number of photometric spikes appear to be lower when mice resided in the recording chamber (data not shown). This observation warrants further investigation, as the chamber used in this study is a common environment in which rodents are housed during microdialysis and other recordings. The nature of the chamber itself -which might be a result of its shape, texture or other unknown factors - could be aversive to animals, particularly under bright light, and thus reduce the amount of DA that can be released in the NAc.

Our results support transfer among familiar, rewarding and novel environments as simple, naturalistic, low-stress method to examine the pattern of activation of DA neurons. Moreover, studies that employ the home cage as a rewarding stimulus have the opportunity to explore motivation in the context of environmental cues more naturalistic than those typically used in other studies of reinforcement. Further experiments are needed to address the question of whether extracellular DA is decreased in the recording chamber and to further evaluate the potential of this simple experimental design to robustly identify the effects of various manipulations on the reward circuitry. Albeit assessment of genetically encoded sensors using fiber photometry provides excellent temporal resolution, absolute quantification of neurotransmitters remains challenging. In future studies, it may be helpful to record DA dynamics in the NAc within each environment using $GRAB_{DA}$ while simultaneously performing high-speed microdialysis to determine the relationship between optical signals and the magnitude of changes in extracellular DA.

Blizard and colleagues speculated that the relatively weak reinforcing effect of “home cage return” reported by Simmons in 1924 might be due to the experimental design in which the experimenter picked up the animal and transferred it into its home cage, whereas in their 2003 study the test animals had direct access to their home cage via the goal box of the maze (Simmons 1924; Blizard et al., 2003). A recent publication examined whether direct handling of the animals confounded the results by Simmons in 1924. They found that rats with direct access to their home cage and food-deprived rats that were motivated by food had

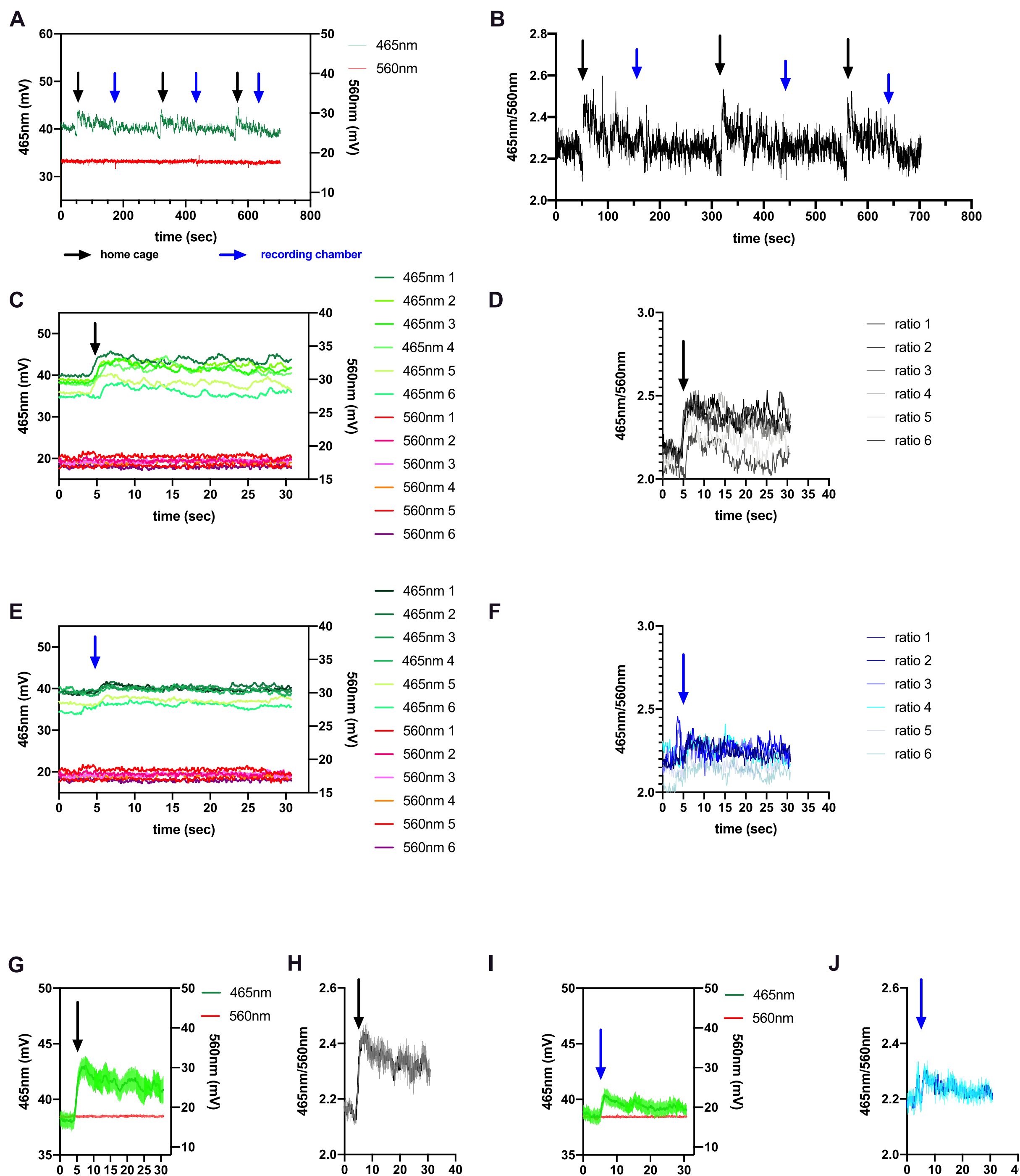

Fig. 2. DA release in the NAc with return to the home cage. (A) Representative time course of repeated transfer of a single mouse, depicting the raw, DA-sensitive signals recorded at 465 nm ($GRAB_{DA}$) and DA-insensitive signals recorded at 560 nm (tdTomato). Black arrows indicate exposure to the home cage. Blue arrows indicate exposure to the recording chamber and the 465nm/560 nm ratio is shown in (B). (C) through (F) display individual 465 nm ($GRAB_{DA}$) and 560 nm (tdTomato) (C,E) recordings and the corresponding ratios (D,F) in response to either the home cage (C and D) or the recording chamber (E and F) at higher temporal resolution and the average is of these traces (mean and standard error of the mean) is shown in G through J.

comparable learning curves for the Lashley III maze and both groups were superior to an indirect home cage group in which an experimenter transferred the rat into its home cage (Taniuchi et al. 2019). Hence, improvements, such as not physically transferring the animal by the tail, given the stress of this procedure (Bressler et al. 2010), can be envisaged, such as lever pressing to access the home cage. Other studies may be able to use this design to identify circuits distinct from those identified to date that connect the mesolimbic reward system to the cognitive awareness of "safety". However, experiments that silence the afferents to the NAc, or the specific cellular targets of dopamine in the NAc, are needed to define the circuitry related to the rewarding aspects of the home cage. Moreover, mutations or early-life or recurrent stressors that drive anhedonic states or depressive-like behavior may benefit from this simple experimental design to identify or optimize stress and mood disorder therapeutic leads. Finally, the availability of other neurotransmitter sensors, such as those for glutamate (Helassa et al., 2018; Marvin et al., 2018), norepinephrine (Feng et al., 2019) and serotonin (Wan et al., 2020), should also allow for the study of other

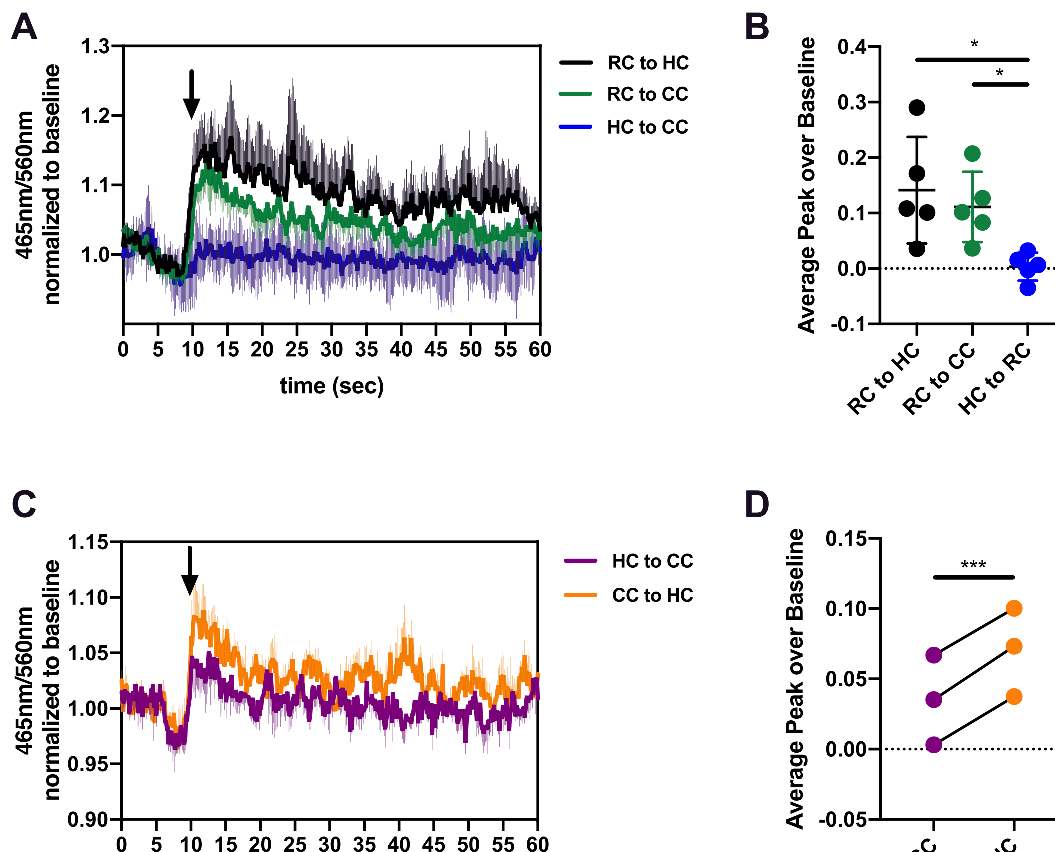

Fig. 3. DA release in the NAc with transfer to home cage, new cage or recording chamber. (A) Ratio of changes in the DA-sensitive signal recorded at 465 nm relative to the DA-insensitive signal recorded at 560 nm upon transfer to the individual environments (indicated by black arrow) (n = 5 mice per group). Abbreviations: Recording chamber to home cage = RC to HC; Recording chamber to clean cage = RC to CC; home cage to recording chamber = HC to RC.(B) Amplitude of the peak in extracellular DA (average of the 465nm/560 nm ratio between t = 11.3 and t = 13.5 s) normalized to the average of the 465nm/560 nm ratio between t = 0 and t = 8.68sec (i.e. baseline) of the recordings shown in (A). Data were analyzed with one-way ANOVA, followed by Dunnett's multiple comparison test; * = $P < 0.05$, n = 5 per group. Line and error bars represent the means and standard deviations, respectively (C) Shown are the ratios of the DA sensitive signal over the DA insensitive signal upon transfer from the home cage to the new cage and *vice versa* (n = 3 mice per group in triplicate). Abbreviations: Home cage to clean cage = HC to CC; clean cage to home cage = CC to HC. (D) Comparison of peak DA (between t = 11.3 and t = 13.5 s) normalized to the average of basal DA (t = 0–8.68sec) of the traces shown in (C) (*** = $P < 0.001$, One-tailed paired Student's t-test, n = 3 per group in triplicate). Data in (A) and (C) are shown as mean and standard error of the mean.

neurochemically-defined inputs to reward centers that make the home environment reinforcing, as for example in communicating the presence of remembered rewards and the relief of anxiety, respectively.

5. Conclusions

By monitoring a well-replicated biomarker associated with reward, NAc DA release, our study provides evidence to support the idea that return to a home cage-like environment is a reinforcing stimulus for mice, consistent with earlier studies demonstrating that access to the home cage or a clean cage that is identical to the home cage promote motivated maze learning (Taniuchi et al. 2019; Blizard et al. 2003, 2006; Bressler et al. 2010). We validate a simple "return to home" manipulation that can be further expanded to explore and manipulate circuits that converge on the mesolimbic reward system to motivate behavior, possibly distinguishing those connected to location and safety versus those often studied in traditional reinforcement tasks (e.g. food or drug reward). Moreover, this simple task may serve as a valuable control for long-term studies that assess DA-release in the NAc in response to rewarding stimuli to ensure for adequate function of the optic probe and expression of genetically encoded DA-sensors. Finally, the amplitude or kinetics of the home return-induced rise in NAc DA may also provide a useful quantitative trait to model disease associated with cognitive, stress and affective disorders.

Author contributions

Felix P Mayer: Planned and designed the study, designed figures, performed all experiments, surgeries and data analysis; interpreted results, wrote and edited the manuscript, Hideki Iwamoto: Assisted with data analysis and provided essential support of fiber photometry studies, Maureen K Hahn: Reviewed and edited the manuscript, Gregory J. Grumbar: Performed Immunohistochemistry, Adele Stewart: Maintained and provided mice, Yulong Li: Provided and advised on use of virally-encoded DA sensors Randy D Blakely: Supervised and supported the studies, edited the manuscript, interpreted results All authors reviewed and approved the manuscript.

Declaration of competing interest

None.

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Appendix A. Supplementary data

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References

Abler, Birgit, Walter, Henrik, Erk, Susanne, Kammerer, Hannes, Spitzer, Manfred, 2006. Prediction error as a linear function of reward probability is coded in human nucleus accumbens. Neuroimage 31 (2), 790–795. https://doi.org/10.1016/j.neuroimage.2006.01.001.

Bayer, Hannah M., Glimcher, Paul W., 2005. Midbrain dopamine neurons encode a quantitative reward prediction error signal. Neuron 47 (1), 129–141. https://doi.org/10.1016/j.neuron.2005.05.020.

Blizard, David A., Laura Cousino, Klein, Rachel, Cohen, McClearn, Gerald E., 2003. A novel mouse-friendly cognitive task suitable for use in aging studies. Behav. Genet. 33 (2), 181–189. https://doi.org/10.1023/A:1022510119598.

Blizard, David A., Weinheimer, Viola K., Klein, Laura Cousino, Petrill, Stephen A., Cohen, Rachel, McClearn, Gerald E., 2006. "'Return to home cage' as a reward for maze learning in young and old genetically heterogeneous mice. Comp. Med. 56 (3), 196–201.

Bressler, Amanda, Blizard, David, Andrews, Anne, 2010. Low-stress route learning using the Lashley iii maze in mice. JoVE 39. https://doi.org/10.3791/1786.

Feng, Jiesi, Zhang, Changmei, Lischinsky, Julieta E., Jing, Miao, Zhou, Jingheng, Wang, Huan, Zhang, Yajun, et al., 2019. A genetically encoded fluorescent sensor for rapid and specific in vivo detection of norepinephrine. Neuron 102 (4), 745–761. https://doi.org/10.1016/j.neuron.2019.02.037 e8.

Ghosal, Sriparna, Nunley, Amanda, Mahbod, Parinaz, Alfor, G., Lewis, Eric P. Smith, Tong, Jenny, D'Alessio, David A., Herman, James P., 2015. Mouse handling limits the impact of stress on metabolic endpoints. Physiol. Behav. 150, 31–37. https://doi.org/10.1016/j.physbeh.2015.06.021.

Helassa, Nordine, Dürst, Céline D., Coates, Catherine, Kerruth, Silke, Arif, Urwa, Schulze, Christian, Simon Wiegert, J., Geeves, Michael, Oertner, Thomas G., Török, Katalin, 2018. Ultrafast glutamate sensors resolve high-frequency release at schaffer collateral synapses. Proc. Natl. Acad. Sci. U. S. A. 115 (21), 5594–5599. https://doi.org/10.1073/pnas.1720648115.

Hernandez, Luis, Hoebel, Bartley G., 1988. Food reward and cocaine increase extracellular dopamine in the nucleus accumbens as measured by microdialysis. Life Sci. 42 (18), 1705–1712. https://doi.org/10.1016/0024-3205(88)90036-7.

Horvitz, J.C., 2000. Mesolimbocortical and nigrostriatal dopamine responses to salient non- reward events. Neuroscience 96 (4), 651–656. https://doi.org/10.1016/S0306-4522(00)00019-1.

Jong, Johannes W. de, Afjei, Seyedeh Atiyeh, Dorocic, Iskra Pollak, Peck, James R., Liu, Christine, Kim, Christina K., Tian, Lin, Deisseroth, Karl, Lammel, Stephan, 2019. A neural circuit mechanism for encoding aversive stimuli in the mesolimbic dopamine system. Neuron 101 (1), 133–151. https://doi.org/10.1016/j.neuron.2018.11.005 e7.

Kristensen, Anders S., Andersen, Jacob, Jorgensen, Trine N., Sorensen, Lena, Jacob, Eriksen, Loland, Claus J., Stromgaard, Kristian, Gether, Ulrik, 2011. SLC6 neurotransmitter transporters: structure, function, and regulation. Pharmacol. Rev. 63 (3), 585–640. https://doi.org/10.1124/pr.108.000869.

Lammel, Stephan, Lim, Byung Kook, Robert, C., Malenka, 2014. Reward and aversion in a heterogeneous midbrain dopamine system. Neuropharmacology 76 (PART B), 351–359. https://doi.org/10.1016/j.neuropharm.2013.03.019.

Marvin, Jonathan S., Benjamin Scholl, Daniel E. Wilson, Podgorski, Kaspar, Kazemipour, Abbas, Müller, Johannes Alexander, Schoch, Susanne, et al., 2018. Stability, affinity, and chromatic variants of the glutamate sensor IGluSnFR. Nat. Methods 15 (11), 936–939. https://doi.org/10.1038/s41592-018-0171-3.

Mayer, Dana, Kahl, Evelyn, Uzuneser, Taygun C., Fendt, Markus, 2018. Role of the mesolimbic dopamine system in relief learning. Neuropsychopharmacology 43 (8), 1651–1659. https://doi.org/10.1038/s41386-018-0020-1.

Myron, Benuck, Abel, Lajtha, Marteen, E., Reith, A., 1987. "J1:". J. Pharmacol. Exp. Therapeut. 243 (1), 144–149.

Navratilova, Edita, Atcherley, Christopher W., Frank, Porreca, 2015. Brain circuits encoding reward from pain relief. Trends Neurosci. 38 (11), 741–750. https://doi.org/10.1016/j.tins.2015.09.003.

Puglisi-Allegra, Stefano, Imperato, Assunta, Angelucci, Luciano, Cabib, Simona, 1991. Acute stress induces time-dependent responses in dopamine mesolimbic system. Brain Res. 554 (1–2), 217–222. https://doi.org/10.1016/0006-8993(91)90192-X.

Rebec, G.V., Grabner, C.P., Johnson, M., Pierce, R.C., Bardo, M.T., 1996. Transient increases in catecholaminergic activity in medial prefrontal cortex and nucleus accumbens shell during novelty. Neuroscience 76 (3), 707–714. https://doi.org/10.1016/S0306-4522(96)00382-X.

Russo, Scott J., Nestler, Eric J., 2013. The brain reward circuitry in mood disorders. Nat. Rev. Neurosci. https://doi.org/10.1038/nrn3381.

Salamone, J.D., Correa, M., Mingote, S.M., Weber, S.M., 2005. Beyond the reward hypothesis: alternative functions of nucleus accumbens dopamine. Curr. Opin. Pharmacol. 5 (1), 34–41. https://doi.org/10.1016/j.coph.2004.09.004.

Simmons, Rietta, 1924. "The relative effectiveness of certain incentives in animal learning. In: Hunter, Walter S. (Ed.), Compar. Psychol. Monogr., 7, 2.

Sun, Fangmiao, Zeng, Jianzhi, Jing, Miao, Zhou, Jingheng, Feng, Jiesi, Owen, Scott F., Luo, Yichen, et al., 2018. A genetically encoded fluorescent sensor enables rapid and specific detection of dopamine in flies, fish, and mice. Cell 174 (2), 481–496. https://doi.org/10.1016/j.cell.2018.06.042 e19.

Sun, Fangmiao, Zhou, Jingheng, Dai, Bing, Qian, Tongrui, Zeng, Jianzhi, Li, Xuelin, Zhuo, Yizhou, et al., 2021. Dopaminergic activity in vivo. Nat. Methods. https://doi.org/10.1038/s41592-020-00981-9.

Taniuchi, Tohru, Ohgi, Akiko, Nishikawa, Mikita, 2019. Returning to home cage serves as an effective reward for maze learning in rats. Behav. Process. 164 (May), 175–177. https://doi.org/10.1016/j.beproc.2019.04.018.

Wan, Jinxia, Peng, Wanling, Li, Xuelin, Qian, Tongrui, Song, Kun, Zeng, Jianzhi, Deng, Fei, et al., 2020. A genetically encoded GRAB sensor for measuring serotonin dynamics in vivo. BioRxiv. https://doi.org/10.1101/2020.02.24.962282, 2020.02.24.962282.

Wise, Roy A., 2004. Dopamine, learning and motivation. Nature Reviews Neuroscience. Nature Publishing Group. https://doi.org/10.1038/nrn1406.

Wise, Roy A., Wang, Bin, You, Zhi Bing, 2008. Cocaine serves as a peripheral interoceptive conditioned stimulus for central glutamate and dopamine release. PloS One 3 (8). https://doi.org/10.1371/journal.pone.0002846.