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A single dose of cocaine raises SV2A density in hippocampus of adolescent rats

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Original Article

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Abstract

Objective Cocaine is a highly addictive psychostimulant that affects synaptic activity with structural and functional adaptations of neurons. The transmembrane synaptic vesicle glycoprotein 2A (SV2A) of pre-synaptic vesicles is commonly used to measure synaptic density, as a novel approach to the detection of synaptic changes. We do not know if a single dose of cocaine suffices to affect pre-synaptic SV2A density, especially during adolescence when synapses undergo intense maturation. Here, we explored potential changes of pre-synaptic SV2A density in target brain areas associated with the cocaine-induced boost of dopaminergic neurotransmission, specifically testing if the effects would last after the return of dopamine levels to baseline. *Methods:* We administered cocaine (20 mg/kg i.p.) or saline to rats in early adolescence, tested their activity levels and removed the brains 1 hour and 7 days after injection. To evaluate immediate and lasting effects, we did autoradiography with [^{3}H]UCB-J, a specific tracer for SV2A, in medial prefrontal cortex, striatum, nucleus accumbens, amygdala, and dorsal and ventral areas of hippocampus. We also measured the striatal binding of [^{3}H]GBR-12935 to test cocaine's occupancy of the dopamine transporter at both times of study. *Results:* We found a significant increase of [^{3}H]UCB-J binding in the dorsal and ventral sections of hippocampus 7 days after the cocaine administration compared to saline-injected rats, but no differences 1 hour after the injection. The [^{3}H]GBR-12935 binding remained unchanged at both times. *Conclusion:* Cocaine provoked lasting changes of hippocampal synaptic SV2A density after a single exposure during adolescence

Significant outcomes

- Single dose of cocaine fails to alter synaptic SV2A density 1 hour after injection
- Single dose of cocaine raises synaptic SV2A density in dorsal and ventral hippocampus 7 days after injection
- Cocaine at a single exposure suffices to provoke lasting synaptic alterations in adolescent rats

Limitations

- We used only adolescent male rats, so we cannot extend the findings to adult or female rats
- We applied a high dose of cocaine (20 mg/kg), leaving it to be determined if lower concentrations would lead to similar findings
- We did not apply additional methods for the assessment of synaptic density in addition to autoradiography
- We included a small sample size (n = 6-8 rats/group)

Introduction

Cocaine is a drug of abuse that can lead to repeated use and addiction in humans (Ward *et al.*, 1997) and animals, including rats (Deroche-Gamonet *et al.*, 2004). As reviewed by Brown and Pollard (Brown and Pollard, 2021), cocaine is well-known for its sympathomimetic psychostimulant action, mediating both behavioural and physical arousal, even at low doses. However, cocaine intoxication has complications that affect almost all organs, in particular the nervous, cardiovascular and respiratory systems (Brown and Pollard, 2021).

The psychostimulant effects of cocaine mainly relate to interactions with monoaminergic pathways, including dopaminergic synapses. Cocaine inhibits dopamine re-uptake by occupying the pre-synaptic dopamine transporters (DAT), thus raising the extracellular neurotransmitter presence above the physiological level and increasing dopaminergic neurotransmission (reviewed, e.g., by Kalivas (2007), Zhu and Reith (2008), Docherty and Alsufyani (2021)). Dopamine receptor occupancy is an important factor in cocaine addiction that tends to be proportional to the degree of cocaine craving (Wong *et al.*, 2006). The cocaine effects on extracellular dopamine are transient, however, as demonstrated by microdialysis (Hurd and Ungerstedt, 1989), intravenous administration of low dose of cocaine produces immediate and dramatic increases of striatal dopamine levels, peaking at 10 minutes after cocaine administration and returning to baseline in 30 minutes.

Cocaine intake also significantly affects neuronal activity by producing important structural and molecular changes in multiple brain areas. Cocaine-induced neuroplasticity has been detected in structures related to dopaminergic neurotransmission and reward mechanisms (reviewed, e.g. in Uys and Reissner (2011), Nyberg (2014)). These adaptations can be variable. For example, changes of N-methyl-D-aspartate receptor signalling induced by cocaine in different structures are heterogeneous and sometimes contradictory, mainly due to the complexity of the signalling pathways and the differences of experimental paradigms associated with cocaine administration, as summarised by Ortinski (Ortinski, 2014). A single dose of cocaine induces long-term potentiation in midbrain dopamine neurons (Ungless *et al.*, 2001), and other studies revealed neuroplastic effects of acute cocaine exposure, indicating molecular, structural and functional changes of the mesolimbic system core (Kozell and Meshul, 2001, Grignaschi *et al.*, 2004, Sarti *et al.*, 2007, Friend *et al.*, 2021).

Adolescence is a sensitive period of life in which synapses undergo major maturation and fine-tuning events, reshaping brain connectivity and morphology with distinct regional variations, particularly in the cortex (Khundrakpam *et al.*, 2016, Juraska and Drzewiecki, 2020). It is plausible that in this period, events that interfere with synaptic function (including drug exposure) significantly influence synaptic maturation. In animals, acute and repeated exposures to cocaine during adolescence cause changes of neurotransmission and neuroplasticity, affect the expression of trophic factors and synaptic elements, and induce immediate or persisting risks to cognition, behaviour and brain development (for review, see Caffino *et al.* (2021)).

The synaptic vesicle glycoprotein 2A (SV2A) commonly reveals synaptic density variations in the brain. Synaptic vesicle glycoproteins 2 constitute a class of transmembrane proteins of all synaptic vesicles (Buckley and Kelly, 1985), and the main isoform SV2A is expressed ubiquitously in all grey matter brain structures, albeit with regional variations (Bajjalieh *et al.*, 1993, Varnäs *et al.*, 2020). SV2A commonly serves as a marker of synaptic density in neuroimaging studies, although some questions remain of the interpretation of the imaging outcomes (Rossi *et al.*, 2022). SV2A seems to have a number of different functions in neurons (for an overview, see Rossi *et al.* (2022)) but SV2A indubitably has crucial roles in the modulation of neurotransmission (Crowder *et al.*, 1999, Bradberry and Chapman, 2022) that make it an interesting target of the study of cocaine addiction. A number of specific tracers of SV2A exist, including UCB-J (Mercier *et al.*, 2014) that proved to be optimally applicable to in vivo neuroimaging by means of positron emission tomography (PET) when radiolabeled with ^{11}C (Nabulsi *et al.*, 2016). *Postmortem* imaging techniques such as autoradiography with [^{3}H]UCB-J also served to assess changes of synaptic SV2A density in preclinical studies (Binda *et al.*, 2021, Raval *et al.*, 2021, Thomsen *et al.*, 2021).

Here, we administered a single high dose of cocaine to rats during early adolescence, with the aim of proving if cocaine has effects on pre-synaptic SV2A density in different brain areas after the cocaine-induced boost of extracellular dopamine. We assessed changes of SV2A density in dopaminergic pathways by evaluating [^{3}H]UCB-J autoradiograms 1 hour and 7 days after cocaine administration. We did [^{3}H]GBR-12935 autoradiography at both times to verify the occupancy of DAT, as a marker of dopaminergic system functionality (as previously described, e.g. by Wong *et al.* (1998)) after the extinction of acute effects of cocaine.

Materials and methods

Animals. We conducted all animal procedures according to the FELASA guidelines for animal experimentation with permission from the Danish Animal Experiment Inspectorate (license number 2016-15-0201-01105) and reported experiments according to the ARRIVE guidelines. We used 28 male Sprague-Dawley rats (Taconic Biosciences, Denmark) of average post-natal day (PND) 35 at the time of experimentation with sacrifice on PND 35 or 42, roughly consistent with the peri-adolescence period (Sengupta, 2013). We chose animals of these ages according to previous literature of cocaine exposure in adolescent rats (Giannotti *et al.*, 2015, Caffino *et al.*, 2017, Caffino *et al.*, 2018, Caffino *et al.*, 2020). Before the experiment, the rats underwent 7 days of environmental habituation in a climate-controlled facility (12 h light/ 12 h dark cycle, average temperature of 22°C, average humidity of 55%) with *ad libitum* access to food and water.

After randomisation, we intraperitoneally injected a single dose of cocaine (20 mg/kg, $N = 16$) or saline ($N = 12$) as control. After the assessment of locomotion parameters to evaluate the success of the treatment, we randomly assigned the rats to two groups, each consisting of 14 animals, including cocaine-injected ($N = 8$) and saline-injected ($N = 6$) rats. We euthanised animals of the two experimental groups by decapitation at different time points, 1 hour or 7 days after single injection. We rapidly removed and froze the whole brains in powdered dry ice and stored them at −80°C.

Open field test (OFT). We performed OFT in a flat and empty arena with a surface of 200 × 200 cm^2, divided into 4 equal quadrants with a surface of 100 × 100 cm^2 each and bordered by walls to prevent interactions between conspecifics during the test. We recorded the entire space of movement of the animals with a camera installed above the arena. We tested the animals in the open field for 30 minutes immediately after the cocaine or saline injection. We analysed the acquired videos by EthoVision XT® (Noldus) software. An observer blind to the treatment groups analysed the mean velocity (cm/s) and distance moved (cm).

Anatomy and cryostat sectioning. We sectioned fresh-frozen whole brains with a cryostat (Cryostar NX70, Thermo Scientific) and identified anatomical regions of interest according to the Paxinos and Watson rat brain atlas (Paxinos and Watson, 2004), including the medial prefrontal cortex (Bregma (B) 3.72 mm, Interaural (I) 12.72 mm), striatum and nucleus accumbens (B 1.80 mm, I 10.80 mm), amygdala (B −2.40 mm, I 6.60 mm), dorsal and ventral hippocampus (B −4.80 mm, I 4.20 mm). We cut the brains along the coronal plane, producing sequential brain sections of 20 µm-thickness. We mounted brain sections on poly-L-lysine coated microscope slides (Thermo

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Scientific). We mounted an average of six brain sections on each slide and stored them at −80°C.

[^{3}H]UCB-J labelling. We thawed the slides at room temperature for 1 hour and then pre-washed them for 15 minutes in Tris-HCl buffer (pH 7.4) containing 50 mM Trizma® base (Sigma) and milliQ water. We divided the slides into two groups to assess total binding (TB) and non-specific (NS) binding. For each animal, we used one slide for TB and one slide for NS for each brain area. We incubated the slides for 1 hour either in TB solution, containing 1 nM [^{3}H]UCB-J (Novandi Chemistry AB; molar activity 78 Ci/mmol) in Tris-HCl buffer, or NS binding solution containing 1 nM [^{3}H]UCB-J and 100 µM levetiracetam (Sigma-Aldrich) in Tris-HCl buffer. We post-washed the slides in Tris-HCl buffer and milliQ water and dried them at room temperature.

[^{3}H]GBR-12935 labelling. We used slides of the striatum to assess the availability of DAT as previously described (Stokholm *et al.*, 2021). For each animal, we used one slide for TB and one slide for NS binding. We thawed the slides at room temperature for 1 hour and then incubated them in a buffer solution containing 50 mM Trizma® base (Sigma), 300 mM NaCl, 0.2% bovine serum albumin (Sigma), 1 µM cis-flupentixol (provided by H. Lundbeck A/S) and milliQ water. We then incubated the slides either in the TB solution containing radiolabeled 2 nM GBR-12935 (PerkinElmer; molar activity 40 Ci/mmol) in the buffer solution or in the NS binding solution containing 2 nM GBR-12935 and 1 µM GBR-12909 (Sigma). We incubated slides overnight at 4°C.

Autoradiography acquisition and analysis. We performed autoradiography acquisitions for 2 hours for [^{3}H]UCB-J and 22 hours for [^{3}H]GBR-12935 with the digital real-time autoradiography BeaQuant system (ai4r, France) (Donnard *et al.* 2009). An experimenter blind to the treatment groups analysed the autoradiograms with Beamage software (version 3.1.2, ai4r, France) using a pixel size of 100 µm. We manually defined regions of interest, respecting the interindividual anatomical differences, and we averaged values from symmetrical brain structures. We subtracted NS binding values (cp/min/mm^2) from TB values (cp/min/mm^2) to obtain specific binding values.

Statistical analysis. We statistically analysed data with GraphPad Prism® 9.4.1. The data passed the Shapiro-Wilk test for normality. We compared experimental groups (saline-treated vs cocaine-treated; sacrifice after 1 hour vs sacrifice after 7 days) with unpaired two-tailed t-tests. We considered p-values <0.05 as indicative of statistical significance, with graphical representation of significance by means of asterisks ((*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). The corresponding author will make complete data collections available upon reasonable request.

Results

Cocaine-treated rats had significantly increased locomotion. The open field test results showed a significant increase in the locomotion measures of cocaine-injected ($N = 16$) compared to saline-injected ($N = 12$) rats. Cocaine-injected rats in particular showed increased mean speed (Fig. 1(a)) (+125.24%; $p = 0.0003$***) and increased distance travelled (Fig. 1(b)) (+123.37%; $p = 0.0003$***) compared to control rats.

We listed the means, standard deviations, p-values and t-values of [^{3}H]UCB-J and [^{3}H]GBR-12935 autoradiography analyses in Table 1. [^{3}H]UCB-J binding was unchanged 1 hour after cocaine administration. Cocaine-injected rats showed no differences of [^{3}H]UCB-J binding compared to control rats in any brain areas analysed 1 hour after the treatment, as shown in medial prefrontal cortex (Fig. 2(a)), striatum (Fig. 2(b)), nucleus accumbens (Fig. 2(c)), amygdala (Fig. 2(d)) and dorsal (Fig. 2(e)) and ventral (Fig. 2(f)) hippocampus. In contrast, [^{3}H]UCB-J binding had increased 7 days after cocaine administration in dorsal (+ 8.96%, $p = 0.0140$*; Fig. 3(e)) and ventral hippocampus (+ 13.17%, $p = 0.0177$*; Fig. 3(f)), compared to control rats. [^{3}H]UCB-J binding remained unchanged in medial prefrontal cortex (Fig. 3(a)), striatum (Fig. 3(b)), nucleus accumbens (Fig. 3(c)) and amygdala (Fig. 3(d)) 7 days after the cocaine injection, compared to saline-injected control rats. The cocaine-injected rats presented no significant changes of striatal [^{3}H]GBR-12935 binding 1 hour (Fig. 4(a)) or 7 days (Fig. 4(b)) after the treatment, compared to control rats.

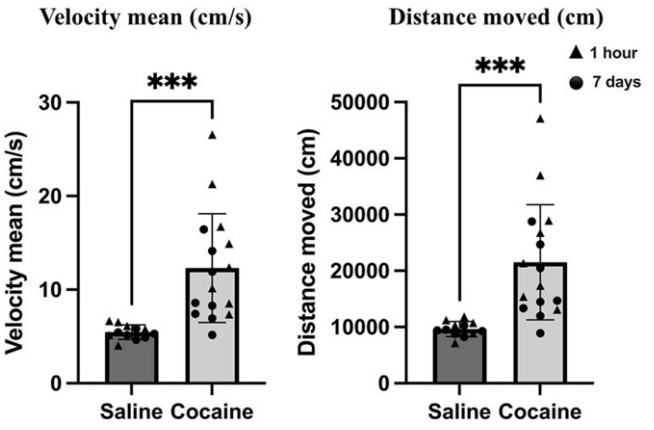

Fig. 1. Open field test results. **(a)** Velocity mean (cm/s) measured in saline-treated ($N = 12$; mean = 5.46 cm/s; SD = 0.77 cm/s) and cocaine-treated ($N = 16$; mean = 12.30 cm/s; SD = 5.82 cm/s) animals; $p < 0.001$***. **(b)** Distance moved (cm) measured in saline-treated ($N = 12$; mean = 9633.90 cm; SD = 1324.61 cm) and cocaine-treated (N = 16; mean = 21,519.42 cm; SD = 10,259.72 cm) animals; $p < 0.001$***. The data are presented as the mean ± SD; SD is graphically shown as vertical bars. Symbols indicate different timepoints for sacrifice (triangles: 1 hour, circles: 7 days).

Discussion

As described, we tested if a single dose of cocaine administered during adolescence alters the density of the SV2A protein, evaluating both short- (1 hour) and long-(7 days) lasting effects. As cocaine raises dopaminergic neurotransmission (Kalivas, 2007, Zhu and Reith, 2008, Docherty and Alsufyani, 2021), and SV2A is involved in different aspects of the modulation of neurotransmission (reviewed in Rossi *et al.* (2022)), we tested the hypothesis that changed SV2A levels in brain areas targeted by dopaminergic pathways eventually would relate to synaptic adaptations incurred by the increased dopamine availability. Previous studies already demonstrated that a single dose of cocaine, administered at the same concentration used in this experimental work (20 mg/kg), is sufficient to elicit molecular, structural (Giannotti *et al.*, 2015, Caffino *et al.*, 2017, Caffino *et al.*, 2018) or behavioural changes (Caffino *et al.*, 2020) in early adolescent rats.

We assessed the successful administration of cocaine by testing the locomotion parameters by OFT. Psychostimulants, like cocaine, induce a sharp increase in dopamine neurotransmission with increased locomotor activity (reviewed, e.g. in Beninger (1983)). We performed the OFT for 30 minutes immediately following the injection as befits the timespan of dopamine raised by the effects of cocaine (Hurd and Ungerstedt, 1989). Cocaine-treated rats moved with elevated mean velocity and distance

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Table 1. [^{3}H]UCB-J and [^{3}H]GBR-12935 autoradiography data. For each analysed brain area, table shows the mean, the standard deviation (± SD), the *p*- and *t*-values for the comparison between cocaine-treated rats vs. saline-treated rats at the two temporal points, 1 hour and 7 days after injection

[^{3}H]UCB-J autoradiography					
Area	Time point	Injection	Average [^{3}H]UCB-J binding (cp/min/mm^2)	Standard deviation	*p*-value, *t*-value
Medial prefrontal cortex	**1 hour**	Saline	20.57	1.71	$p = 0.1282$ $t = 1.634$
		Cocaine	19.08	1.66	
	7 days	Saline	18.60	1.59	$p = 0.4236$ $t = 0.828$
		Cocaine	19.57	2.50	
Striatum	**1 hour**	Saline	12.92	1.48	$p = 0.2740$ $t = 1.146$
		Cocaine	11.79	2.04	
	7 days	Saline	11.44	1.29	$p = 0.9179$ $t = 0.1053$
		Cocaine	11.33	2.27	
Nucleus accumbens	**1 hour**	Saline	14.99	2.45	$p = 0.6368$ $t = 0.485$
		Cocaine	14.35	2.44	
	7 days	Saline	13.56	3.05	$p = 0.8520$ $t = 0.191$
		Cocaine	13.85	2.66	
Amygdala	**1 hour**	Saline	14.08	2.51	$p = 0.2715$ $t = 1.153$
		Cocaine	12.39	2.87	
	7 days	Saline	12.39	1.28	$p = 0.0651$ $t = 2.030$
		Cocaine	14.31	2.02	
Dorsal hippocampus	**1 hour**	Saline	14.54	1.40	$p = 0.5737$ $t = 0.578$
		Cocaine	14.14	1.14	
	7 days	Saline	13.83	0.57	$p = 0.0140$ (*) $t = 2.873$
		Cocaine	15.07	0.93	
Ventral hippocampus	**1 hour**	Saline	14.18	2.23	$p = 0.3497$ $t = 0.973$
		Cocaine	13.32	0.99	
	7 days	Saline	13.62	1.02	$p = 0.0177$ (*) $t = 2.787$
		Cocaine	15.42	1.19	
[^{3}H]GBR-12935 autoradiography					
Area	Time point	Injection	Average [^{3}H]GBR-12935 binding (cp/min/mm^2)	Standard deviation	*p*-value, *t*-value
Striatum	**1 hour**	Saline	0.62	0.09	$p = 0.8557$ $t = 0.186$
		Cocaine	0.63	0.15	
	7 days	Saline	0.78	0.13	$p = 0.2943$ $t = 1.097$
		Cocaine	0.70	0.15	

compared to control rats. The cocaine-induced increase of locomotor activity previously was reported in the literature (e.g. Yeh and Haertzen (1991)), and early adolescence rats have been shown to have the greatest rise of motor activity following cocaine administration, compared to rats of other ages (Badanich *et al.*, 2008).

We verified the activity of the dopaminergic system by means of [^{3}H]GBR-12935 autoradiography, showing that striatal [^{3}H]GBR-12935 binding was unchanged in cocaine-treated rats compared to controls at both times of analysis. Cocaine persistency in the dopaminergic system has been assessed by different studies; for example, Hurd and Ungerstedt (1989) showed that the striatal cocaine-induced rise of dopamine returns to baseline within 30 minutes from the administration. However, another study (Javaid and Davis, 1993) demonstrated that cocaine levels remain high 1 hour after intraperitoneal injection in different rat brain areas, meaning that cocaine occupancy of DAT may be expected at this time. The present [^{3}H]GBR-12935 autoradiography results suggest that at 1 hour, and 7 days after the treatment, cocaine no longer occupies DAT in the striatum and that dopaminergic transmission is normal at both times.

The [^{3}H]UCB-J autoradiography results imply no significant changes 1 hour after the treatment, but the SV2A density of cocaine-injected rats had increased compared to control animals 7 days after the administration in dorsal and ventral hippocampus (Table 1). It is noteworthy also to mention the trend towards an increase in SV2A density in amygdala 7 days after the treatment

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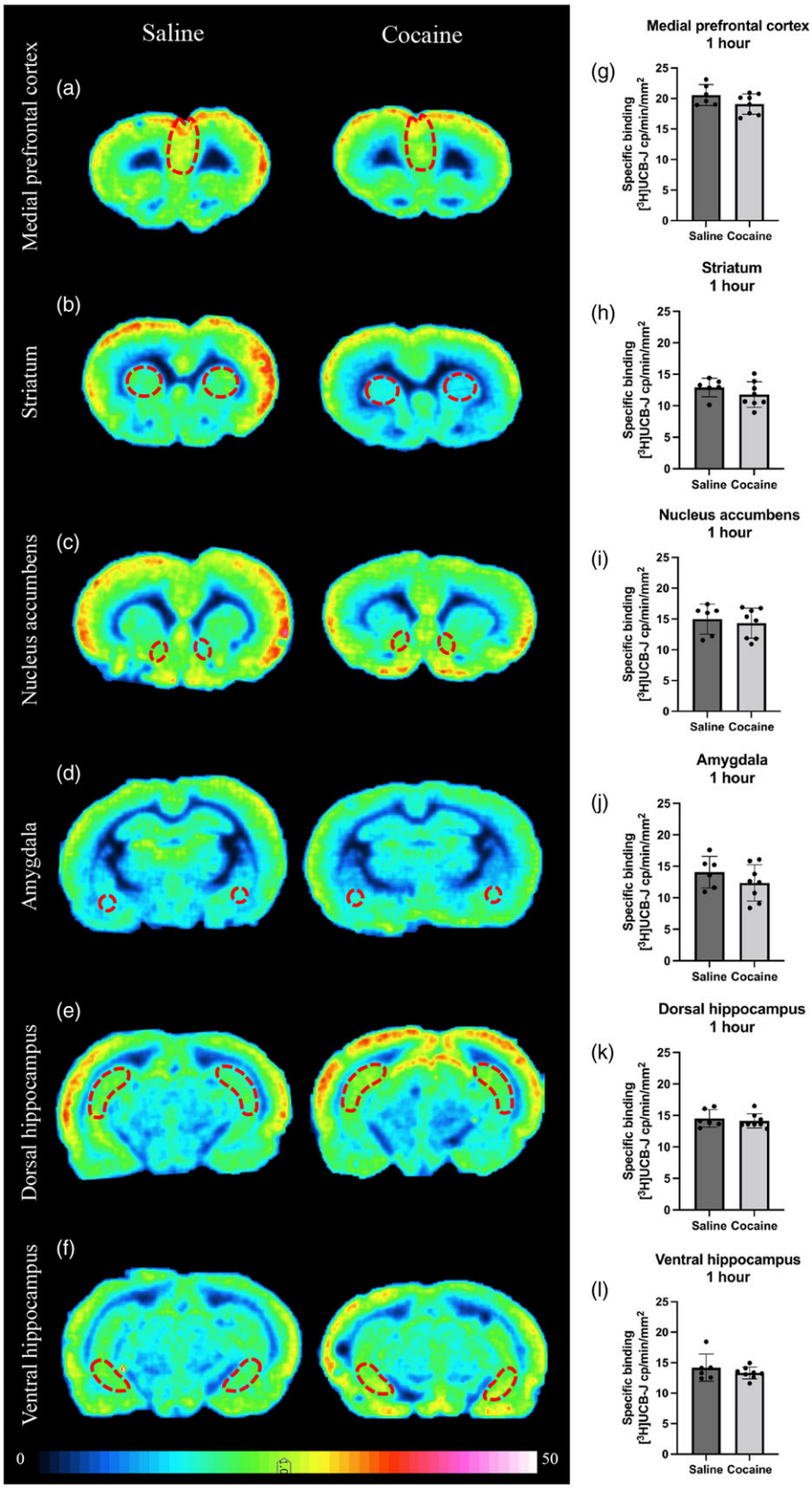

Fig. 2. Effects of a single dose of cocaine on SV2A density 1 hour after the treatment. Representative autoradiograms of [^{3}H]UCB-J total binding in saline-treated and cocaine-treated rats 1 hour after the treatment in **(a)** medial prefrontal cortex, **(b)** striatum, **(c)** nucleus accumbens, **(d)** amygdala, **(e)** dorsal hippocampus and **(f)** ventral hippocampus. The scale bar represents the number of radioactive disintegrations. No changes in [^{3}H]UCB-J specific binding were detected. Comparison of [^{3}H]UCB-J specific binding in saline-treated ($N = 6$) vs. cocaine-treated ($N = 8$) rats 1 hour after the administration in **(g)** medial prefrontal cortex, **(h)** striatum, **(i)** nucleus accumbens, **(j)** amygdala, **(k)** dorsal hippocampus and **(l)** ventral hippocampus. The data are presented as the mean ± standard deviation (SD); SD is graphically shown as vertical bars.

(Table 1). These data suggest that a single administration of cocaine can provoke changes of SV2A density that are evident at least one week after the exposure, much beyond the acute effects of the drug. Since SV2A does not identify a specific agent of neurotransmission (Buckley and Kelly, 1985), it is difficult to associate the [^{3}H]UCB-J autoradiography outcomes with specific types of

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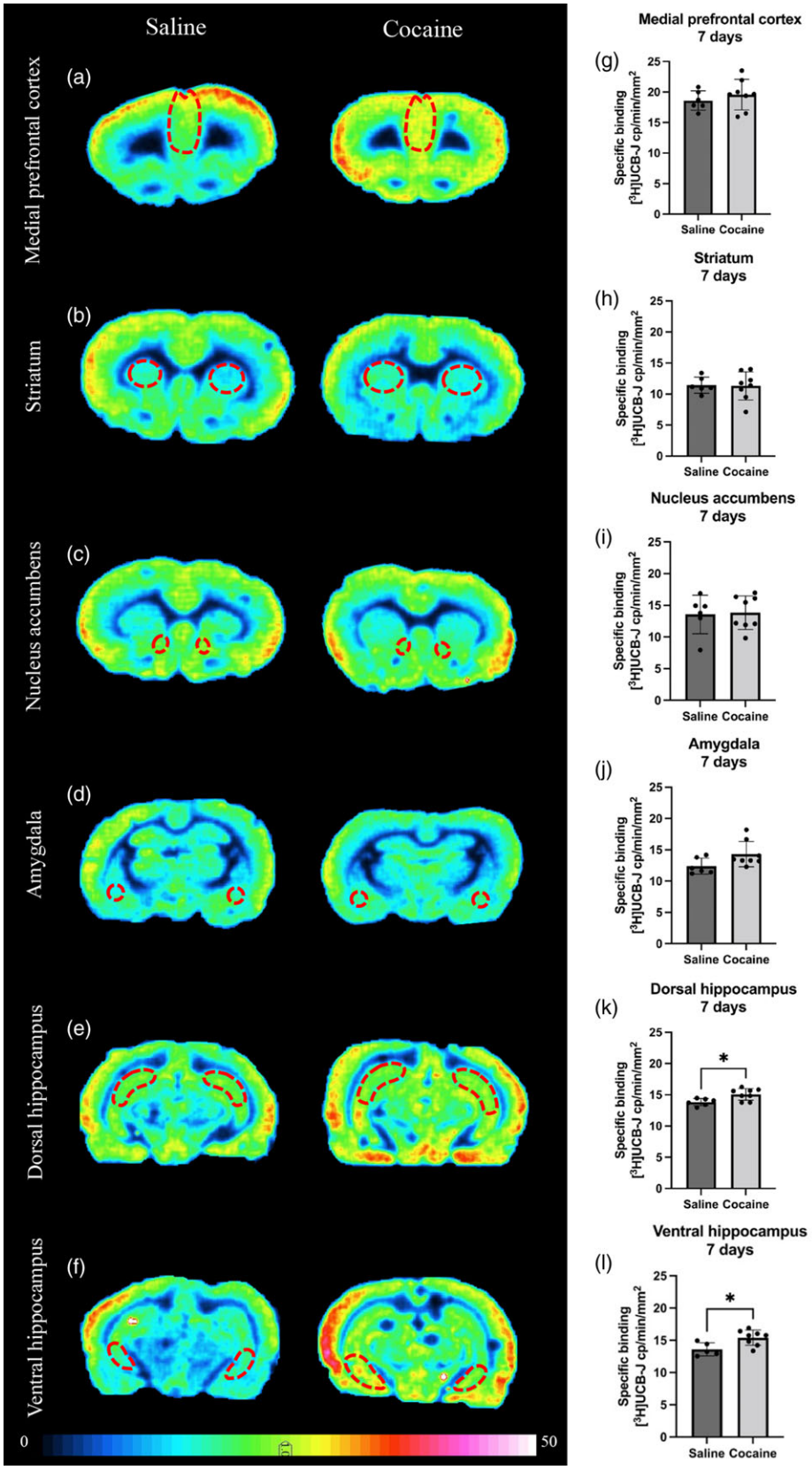

Fig. 3. Effects of a single dose of cocaine on SV2A density 7 days after the treatment. Representative autoradiograms of [^{3}H]UCB-J total binding in saline-treated and cocaine-treated rats 7 days after the treatment in **(a)** medial prefrontal cortex, **(b)** striatum, **(c)** nucleus accumbens, **(d)** amygdala, **(e)** dorsal hippocampus and **(f)** ventral hippocampus. The scale bar represents the number of radioactive disintegrations. Changes in [^{3}H]UCB-J specific binding were detected in dorsal and ventral hippocampus. Comparison of [^{3}H]UCB-J specific binding in saline-treated ($N = 6$; $N = 5$ for ventral hippocampus) vs. cocaine-treated ($N = 8$) rats 7 days after the administration in **(g)** medial prefrontal cortex, **(h)** striatum, **(i)** nucleus accumbens, **(j)** amygdala, **(k)** dorsal hippocampus ($p < 0.05$*) and **(l)** ventral hippocampus ($p < 0.05$*). The data are presented as the mean ± standard deviation (SD); SD is graphically shown as vertical bars.

neurons and specific phenomena. However, cocaine is known to be associated with molecular and functional changes in synapses; for example, cocaine-facilitated neuroplasticity has been observed in several brain structures, including amygdala (e.g. Goussakov *et al.* (2006), Fu *et al.* (2007)) and hippocampus (e.g. Thompson *et al.* (2002), Thompson *et al.* (2004), Thompson *et al.* (2005),

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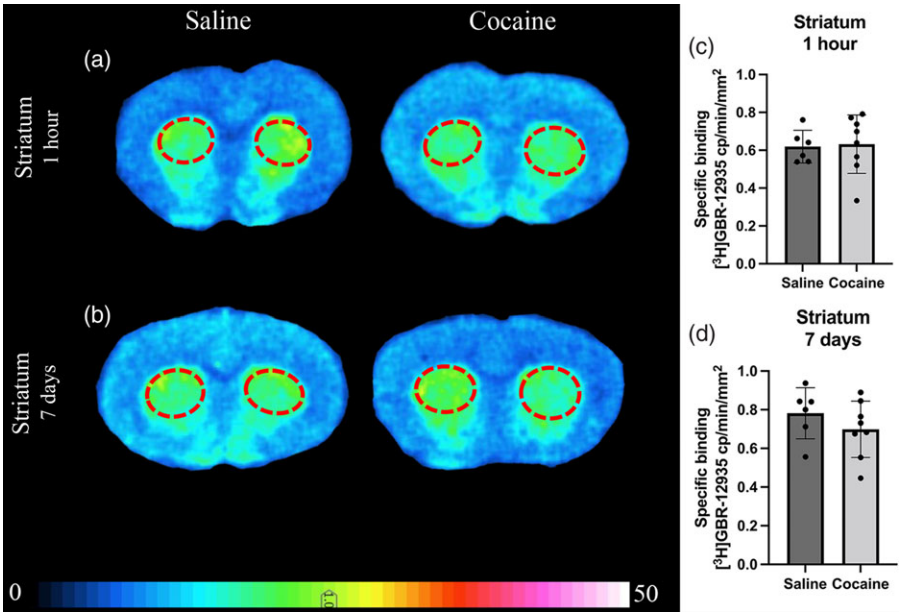

Fig. 4. Effects of a single dose of cocaine on DAT occupancy 1 hour and 7 days after the treatment. Representative autoradiograms of [^{3}H]GBR-12935 total binding in **(a)** striatum of saline-treated vs. cocaine-treated rats 1 hour after the treatment and **(b)** striatum of saline-treated vs. cocaine-treated rats 7 days after the treatment. The scale bar represents the number of radioactive disintegrations. No changes in [^{3}H]GBR-12935 specific binding were detected. **(c)** Comparison of [^{3}H]GBR-12935 specific binding in the striatum of saline- ($N = 6$) and cocaine-injected ($N = 8$) animals 1 hour after the administration. **(d)** Comparison of [^{3}H]GBR-12935 binding in the striatum of saline- ($N = 6$) and cocaine-injected ($N = 8$) animals 7 days after the administration. The data are presented as the mean ± SD; SD is graphically shown as vertical bars.

del Olmo *et al.* (2006), Keralapurath *et al.* (2014), Keralapurath *et al.* (2017)), with different protocols of drug exposure and using different techniques. Some studies focused on the effects of cocaine in the dorsal and ventral hippocampus (e.g. Keralapurath *et al.* (2014), Keralapurath *et al.* (2017), Preston *et al.* (2019), Werner *et al.* (2020), Qi *et al.* (2022)), with varying synaptic alterations observed in these areas and across the different studies. Therefore, we suggest the increased hippocampal SV2A density to be linked with cocaine-induced changes in synapses which may occur after a single drug exposure. However, to our knowledge, specific studies of the contribution of SV2A to drug-facilitated neuroplastic processes are lacking.

The involvement of the hippocampus in onset, development and maintenance of addiction through drug-associated memory formation has already been hypothesised and investigated (reviewed by Kutlu and Gould (2016)). So, the presence of cocaine-induced changes in hippocampal synapses after a single dose may be linked with the formation of drug-associated memories and may relate to the onset of addiction in adolescence. However, we did not investigate long-term behavioural effects in the present study, so further studies are necessary to fully understand the particular molecular events that are attributable to these changes, and how SV2A may participate in cocaine-induced neuroadaptations. Comparisons with adult animals are also necessary to clarify if these effects are related to adolescence or if they extend to adulthood.

The lack of changes in [^{3}H]UCB-J binding in the medial prefrontal cortex of cocaine-injected rats compared to controls is worth a comment. The literature is equivocal about the effects of cocaine in this region. For example, some studies (e.g. Robinson and Kolb (1999), Muñoz-Cuevas *et al.* (2013)) reported increased dendritic spine density in rodent frontal cortex after repeated cocaine exposure. Caffino *et al.* (2018) showed that a single dose of cocaine with the same concentration of 20 mg/kg used here provoked a decrease of dendritic spine density and an impairment of post-synaptic elements of glutamatergic terminals in the medial prefrontal cortex of adolescent rats aged 35 days at the time of the injection, with effects detected 7 days after the drug exposure. A PET study (Angarita *et al.*, 2022) of SV2A density in the medial prefrontal cortex of patients with cocaine use disorder revealed decreased [^{11}C]UCB-J binding in frontal cortices, but in this work, the subjects had a history of repeated cocaine exposures, different from the present experimental setup in which we analysed the effects of a single dose in rat. Overall, further studies are necessary to clarify if and how acute cocaine effectively can modify synapses of the medial prefrontal cortex with short- or long-term effects.

In conclusion, the present experimental work investigated both the immediate and lasting effects of a single high dose of cocaine on a measure of pre-synaptic SV2A density in male adolescent rats by using [^{3}H]UCB-J autoradiography. The results revealed an increased tracer binding in the dorsal and ventral hippocampus 7 days after the injection of cocaine compared to saline-injected rats. We emphasised the possible importance of the detected synaptic changes in hippocampus as possible early indicators of cocaine addiction development in adolescents, but further studies are needed to understand the ultimate causes of such changes.

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References

Angarita GA, Worhunsky PD, Naganawa M, Toyonaga T, Nabulsi NB, Li CR, Esterlis I, Skosnik PD, Radhakrishnan R, Pittman B, Gueorguieva R, Potenza MN, Finnema SJ, Huang Y, Carson RE, Malison RT (2022) Lower prefrontal cortical synaptic vesicle binding in cocaine use disorder: an exploratory (11) C-UCB-J positron emission tomography study in humans. *Addiction Biology* **27**(2), e13123.

Badanich KA, Maldonado AM and Kirstein CL (2008) Early adolescents show enhanced acute cocaine-induced locomotor activity in comparison to late adolescent and adult rats. *Developmental Psychobiology* **50**, 127–133.

Bajjalieh SM, Peterson K, Linial M and Scheller RH (1993) Brain contains two forms of synaptic vesicle protein 2. *Proceedings of the National Academy of Sciences* **90**(6), 2150–2154.

Beninger RJ (1983) The role of dopamine in locomotor activity and learning. *Brain Research* **287**(2), 173–196.

Binda KH, Lillethorup TP, Real CC, Bærentzen SL, Nielsen MN, Orlowski D, Brooks DJ, Chacur M and Landau AM (2021) Exercise protects synaptic density in a rat model of Parkinson's disease. *Experimental Neurology* **342**, 113741.

Bradberry MM and Chapman ER (2022) All-optical monitoring of excitation-secretion coupling demonstrates that SV2A functions downstream of evoked Ca(2+) entry. *The Journal of Physiology* **600**(3), 645–654.

Brown H and Pollard KA (2021) Drugs of abuse: sympathomimetics. *Critical Care Clinics* **37**(3), 487–499.

Buckley K and Kelly RB (1985) Identification of a transmembrane glycoprotein specific for secretory vesicles of neural and endocrine cells. *Journal of Cell Biology* **100**(4), 1284–1294.

Caffino L, Giannotti G, Racagni G and Fumagalli F (2017) A single cocaine exposure disrupts actin dynamics in the cortico-accumbal pathway of adolescent rats: modulation by a second cocaine injection. *Psychopharmacology (Berl)* **234**(8), 1217–1222.

Caffino L, Messa G and Fumagalli F (2018) A single cocaine administration alters dendritic spine morphology and impairs glutamate receptor synaptic retention in the medial prefrontal cortex of adolescent rats. *Neuropharmacology* **140**, 209–216.

Caffino L, Mottarlini F, Mingardi J, Zita G, Barbon A and Fumagalli F (2020) Anhedonic-like behavior and BDNF dysregulation following a single injection of cocaine during adolescence. *Neuropharmacology* **175**, 108161.

Caffino L, Mottarlini F, Zita G, Gawliński D, Gawlińska K, Wydra K, Przegaliński E and Fumagalli F (2021) The effects of cocaine exposure in adolescence: behavioural effects and neuroplastic mechanisms in experimental models. *British Journal of Pharmacology* **179**(17), 4233–4253.

Crowder KM, Gunther JM, Jones TA, Hale BD, Zhang HZ, Peterson MR, Scheller RH, Chavkin C and Bajjalieh SM (1999) Abnormal neurotransmission in mice lacking synaptic vesicle protein 2A (SV2A). *Proceedings of the National Academy of Sciences* **96**(26), 15268–15273.

Del Olmo N, Miguéns M, Higuera-Matas A, Torres I, García-Lecumberri C, Solís JM and Ambrosio E (2006) Enhancement of hippocampal long-term potentiation induced by cocaine self-administration is maintained during the extinction of this behavior. *Brain Research* **1116**(1), 120–126.

Deroche-Gamonet V, Belin D and Piazza PV (2004) Evidence for addiction-like behavior in the rat. *Science* **305**(5686), 1014–1017.

Docherty JR and Alsufyani HA (2021) Pharmacology of drugs used as stimulants. *Journal of Clinical Pharmacology* **61**(Suppl 2), S53–s69.

Donnard J, Thers D, Servagent N and Luquin L (2009) High spatial resolution in β-imaging with a PIM device. *IEEE Transactions on Nuclear Science* **56**(1), 197–200.

Friend LN, Wu B and Edwards JG (2021) Acute cocaine exposure occludes long-term depression in ventral tegmental area GABA neurons. *Neurochemistry International* **145**, 105002.

Fu Y, Pollandt S, Liu J, Krishnan B, Genzer K, Orozco-Cabal L, Gallagher JP and Shinnick-Gallagher P (2007) Long-term potentiation (LTP) in the central amygdala (CeA) is enhanced after prolonged withdrawal from chronic cocaine and requires CRF1 receptors. *Journal of Neurophysiology* **97**(1), 937–941.

Giannotti G, Caffino L, Malpighi C, Melfi S, Racagni G and Fumagalli F (2015) A single exposure to cocaine during development elicits regionally-selective changes in basal basic Fibroblast Growth Factor (FGF-2) gene expression and alters the trophic response to a second injection. *Psychopharmacology (Berl)* **232**(4), 713–719.

Goussakov I, Chartoff EH, Tsvetkov E, Gerety LP, Meloni EG, Carlezon WA Jr. and Bolshakov VY (2006) LTP in the lateral amygdala during cocaine withdrawal. *European Journal of Neuroscience* **23**(1), 239–250.

Grignaschi G, Burbassi S, Zennaro E, Bendotti C and Cervo L (2004) A single high dose of cocaine induces behavioural sensitization and modifies mRNA encoding GluR1 and GAP-43 in rats. *European Journal of Neuroscience* **20**(10), 2833–2837.

Hurd YL and Ungerstedt U (1989) Cocaine: an in vivo microdialysis evaluation of its acute action on dopamine transmission in rat striatum. *Synapse* **3**, 48–54.

Javaid JI and Davis JM (1993) Cocaine disposition in discrete regions of rat brain. *Biopharmaceutics & Drug Disposition* **14**, 357–364.

Juraska JM and Drzewiecki CM (2020) Cortical reorganization during adolescence: what the rat can tell us about the cellular basis. *Developmental Cognitive Neuroscience* **45**, 100857.

Kalivas PW (2007) Neurobiology of cocaine addiction: implications for new pharmacotherapy. *American Journal on Addictions* **16**(2), 71–78.

Keralapurath MM, Briggs SB and Wagner JJ (2017) Cocaine self-administration induces changes in synaptic transmission and plasticity in ventral hippocampus. *Addiction Biology* **22**(2), 446–456.

Keralapurath MM, Clark JK, Hammond S and Wagner JJ (2014) Cocaine- or stress-induced metaplasticity of LTP in the dorsal and ventral hippocampus. *Hippocampus* **24**(5), 577–590.

Khundrakpam BS, Lewis JD, Zhao L, Chouinard-Decorte F and Evans AC (2016) Brain connectivity in normally developing children and adolescents. *Neuroimage* **134**, 192–203.

Kozell LB and Meshul CK (2001) The effects of acute or repeated cocaine administration on nerve terminal glutamate within the rat mesolimbic system. *Neuroscience* **106**(1), 15–25.

Kutlu MG and Gould TJ (2016) Effects of drugs of abuse on hippocampal plasticity and hippocampus-dependent learning and memory: contributions to development and maintenance of addiction. *Learning & Memory* **23**(10), 515–533.

Mercier J, Archen L, Bollu V, Carré S, Evrard Y, Jnoff E, Kenda B, Lallemand B, Michel P, Montel F, Moureau F, Price N, Quesnel Y, Sauvage X, Valade A, Provins L (2014) Discovery of heterocyclic nonacetamide synaptic vesicle protein 2A (SV2A) ligands with single-digit nanomolar potency: opening avenues towards the first SV2A positron emission tomography (PET) ligands. *ChemMedChem* **9**(4), 693–698.

Muñoz-Cuevas FJ, Athilingam J, Piscopo D and Wilbrecht L (2013) Cocaine-induced structural plasticity in frontal cortex correlates with conditioned place preference. *Nature Neuroscience* **16**(10), 1367–1369.

Nabulsi NB, Mercier J, Holden D, Carré S, Najafzadeh S, Vandergeten MC, Lin SF, Deo A, Price N, Wood M, Lara-Jaime T, Montel F, Laruelle M, Carson RE, Hannestad J, Huang Y (2016) Synthesis and preclinical evaluation of 11C-UCB-J as a PET tracer for imaging the synaptic vesicle glycoprotein 2A in the brain. *Journal of Nuclear Medicine* **57**(5), 777–784.

Nyberg F (2014) Structural plasticity of the brain to psychostimulant use. *Neuropharmacology* **87**, 115–124.

Ortinski PI (2014) Cocaine-induced changes in NMDA receptor signaling. *Molecular Neurobiology* **50**(2), 494–506.

Paxinos G and Watson C (2004) The rat brain in stereotaxic coordinates, 5th edn. Elsevier Academic Press.

Preston CJ, Brown KA and Wagner JJ (2019) Cocaine conditioning induces persisting changes in ventral hippocampus synaptic transmission, long-term potentiation, and radial arm maze performance in the mouse. *Neuropharmacology* **150**, 27–37.

Qi S, Tan SM, Wang R, Higginbotham JA, Ritchie JL, Ibarra CK, Arguello AA, Christian RJ and Fuchs RA (2022) Optogenetic inhibition of the dorsal hippocampus CA3 region during early-stage cocaine-memory reconsolidation disrupts subsequent context-induced cocaine seeking in rats. *Neuropsychopharmacology* **47**(8), 1473–1483.

Raval NR, Johansen A, Donovan LL, Ros NF, Ozenne B, Hansen HD and Knudsen GM (2021) A single dose of psilocybin increases synaptic density

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and decreases 5-HT(2A) receptor density in the pig brain. *International Journal of Molecular Sciences* **22**(2), 835. doi:10.3390/ijms22020835.

Robinson TE and Kolb B (1999) Alterations in the morphology of dendrites and dendritic spines in the nucleus accumbens and prefrontal cortex following repeated treatment with amphetamine or cocaine. *European Journal of Neuroscience* **11**(5), 1598–1604.

Rossi R, Arjmand S, Bærentzen SL, Gjedde A and Landau AM (2022) Synaptic vesicle glycoprotein 2A: features and functions. *Frontiers in Neuroscience* **16**, 864514.

Sarti F, Borgland SL, Kharazia VN and Bonci A (2007) Acute cocaine exposure alters spine density and long-term potentiation in the ventral tegmental area. *European Journal of Neuroscience* **26**(3), 749–756.

Sengupta P (2013) The laboratory rat: relating its age with human's. *International Journal of Preventive Medicine* **4**, 624–630.

Stokholm K, Thomsen MB, Phan JA, Møller LK, Bay-Richter C, Christiansen SH, Woldbye DPD, Romero-Ramos M and Landau AM (2021) α-Synuclein overexpression increases dopamine D2/3 receptor binding and immune activation in a model of early Parkinson's disease. *Biomedicines* **9**(12), 1876.

Thompson AM, Gosnell BA and Wagner JJ (2002) Enhancement of long-term potentiation in the rat hippocampus following cocaine exposure. *Neuropharmacology* **42**(8), 1039–1042.

Thompson AM, Swant J, Gosnell BA and Wagner JJ (2004) Modulation of long-term potentiation in the rat hippocampus following cocaine self-administration. *Neuroscience* **127**(1), 177–185.

Thompson AM, Swant J and Wagner JJ (2005) Cocaine-induced modulation of long-term potentiation in the CA1 region of rat hippocampus. *Neuropharmacology* **49**(2), 185–194.

Thomsen MB, Jacobsen J, Lillethorup TP, Schacht AC, Simonsen M, Romero-Ramos M, Brooks DJ and Landau AM (2021) In vivo imaging of synaptic SV2A protein density in healthy and striatal-lesioned rats with [11C]UCB-J PET. *Journal of Cerebral Blood Flow & Metabolism* **41**(4), 819–830.

Ungless MA, Whistler JL, Malenka RC and Bonci A (2001) Single cocaine exposure in vivo induces long-term potentiation in dopamine neurons. *Nature* **411**(6837), 583–587.

Uys JD and Reissner KJ (2011) Glutamatergic neuroplasticity in cocaine addiction. *Progress in Molecular Biology and Translational Science* **98**, 367–400.

Varnäs K, Stepanov V and Halldin C (2020) Autoradiographic mapping of synaptic vesicle glycoprotein 2A in non-human primate and human brain. *Synapse* **74**, e22157.

Ward AS, Haney M, Fischman MW and Foltin RW (1997) Binge cocaine self-administration in humans: intravenous cocaine. *Psychopharmacology (Berl)* **132**(4), 375–381.

Werner CT, Mitra S, Auerbach BD, Wang ZJ, Martin JA, Stewart AF, Gobira PH, Iida M, An C, Cobb MM, Caccamise A, Salvi RJ, Neve RL, Gancarz AM, Dietz DM (2020) Neuroadaptations in the dorsal hippocampus underlie cocaine seeking during prolonged abstinence. *Proceedings of the National Academy of Sciences* **117**(42), 26460–26469.

Wong DF, Kuwabara H, Schretlen DJ, Bonson KR, Zhou Y, Nandi A, Brasic JR, Kimes AS, Maris MA, Kumar A, Contoreggi C, Links J, Ernst M, Rousset O, Zukin S, Grace AA, Lee JS, Rohde C, Jasinski DR, Gjedde A and London ED (2006) Increased occupancy of dopamine receptors in human striatum during cue-elicited cocaine craving. *Neuropsychopharmacology* **31**(12), 2716–2727.

Wong DF, Ricaurte G, Gründer G, Rothman R, Naidu S, Singer H, Harris J, Yokoi F, Villemagne V, Szymanski S, Gjedde A, Kuhar M (1998) Dopamine transporter changes in neuropsychiatric disorders. *Advances in Pharmacology* **42**, 219–223.

Yeh SY and Haertzen CA (1991) Cocaine-induced locomotor activity in rats. *Pharmacology Biochemistry and Behavior* **39**(3), 723–727.

Zhu J and Reith ME (2008) Role of the dopamine transporter in the action of psychostimulants, nicotine, and other drugs of abuse. *CNS & Neurological Disorders - Drug Targets* **7**(5), 393–409.

https://doi.org/10.1017/neu.2023.14 Published online by Cambridge University Press