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Glucocorticoid interactions with the dorsal striatal endocannabinoid system in regulating inhibitory avoidance memory

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ABSTRACT

The endocannabinoid (eCB) system is highly stress sensitive and known to modulate memory formation of emotionally arousing experiences across different corticolimbic structures. eCB signaling within these circuits is also essentially involved in regulating non-genomically mediated glucocorticoid hormone effects on memory. It has long been thought that the dorsal striatum, which plays a major role in procedural memory and habit formation, is considerably less impacted by stressful experiences; however, recent findings indicate that stress and glucocorticoids also affect striatal-dependent memory processes. Yet, to what extent eCB signaling within the dorsal striatum may mediate such glucocorticoid effects on memory consolidation is currently unknown. Here we show, in male Wistar rats, that the cannabinoid agonist WIN55,212-2 administered into the dorsal striatum immediately after an inhibitory avoidance training experience dose-dependently enhanced 48-h retention performance. Conversely, the cannabinoid type 1 receptor (CB1R) antagonist AM251 impaired retention when administered into the dorsal striatum after inhibitory avoidance training. Most importantly, antagonism of striatal CB1R activity with AM251 completely abolished the effect of corticosterone or of the membrane-impermeable ligand corticosterone:BSA administered posttraining into the dorsal striatum or injected systemically on enhancement of inhibitory avoidance memory. Further, suppression of glucocorticoid signaling by systemic injection of the corticosterone-synthesis inhibitor metyrapone also impaired the memory-enhancing effect of intra-striatal WIN55, 212-2 administration. These findings indicate that the eCB system, in close interaction with glucocorticoid signaling, is involved in modulating plasticity changes underlying memory consolidation not only in corticolimbic structures but also within the dorsal striatum.

1. Introduction

The endocannabinoid (eCB) system, mainly through activity-dependent retrograde actions of the endogenous ligands anandamide and 2-arachidonoylglycerol on cannabinoid type 1 receptors (CB1Rs), controls the firing rate of both excitatory and inhibitory neurons, which is vital for neural communication and plasticity changes (Di Marzo et al., 1998; Gray et al., 2014; Lovinger and Mathur, 2017; Ohno-Shosaku and Kano, 2014). The eCB system is highly sensitive to stress and emerged as a key modulator of the stress response, emotion regulation and emotional memory (Balsevich et al., 2017; Hill et al., 2010b; Lutz et al., 2015; Morena et al., 2016). For example, the eCB system plays a crucial role in regulating fast glucocorticoid feedback actions on hypothalamic-pituitary-adrenocortical axis activity (Di et al., 2003; Di and Tasker, 2008; Hill et al., 2010a; Hillard et al., 2016). eCBs also regulate anxiety-like behaviors, either preventing or prompting anxiety depending on the dose and experimental condition (Lutz et al., 2015; Rey et al., 2012).

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Glucocorticoid hormones (corticosterone in rodents, cortisol in humans) are known to strengthen the consolidation of long-term memory of emotionally arousing experiences in rodents (de Kloet et al., 1999; McGaugh and Roozendaal, 2002; Okuda et al., 2004; Roozendaal and McGaugh, 2011; Sandi and Pinelo-Nava, 2007) and humans (Buchanan and Lovallo, 2001; Kuhlmann and Wolf, 2006). Several findings indicate that stress and glucocorticoid hormones induce fast changes in eCB signaling in corticolimbic areas (Hill et al., 2010a, 2011). Aversively motivated inhibitory avoidance training also induces a rapid increase in anandamide levels within corticolimbic circuits (Morena et al., 2014), whereas a blockade of CB1R activity in the amygdala or hippocampus abolishes glucocorticoid-induced enhancement of inhibitory avoidance memory (Atsak et al., 2015; Campolongo et al., 2009). These findings indicating that glucocorticoids essentially interact with the eCB system in enhancing consolidation processes (Atsak et al., 2015; Campolongo et al., 2009; Hill and McEwen, 2009; Morena et al., 2014) reiterate the growing notion of the eCB system as a rapid mediator of responses to stress and stress hormones (Balsevich et al., 2017).

The predominant view held that stress might have a particularly robust influence on corticolimbic structures (Kim et al., 2006; Lupien and McEwen, 1997; McEwen and Sapolsky, 1995; Roozendaal et al., 2009) whereas the dorsal striatum, which is involved in procedural memory and habit formation, is relatively spared (Goodman et al., 2012; Schwabe and Wolf, 2013). This view has been supported by findings of studies investigating the modulatory effects of stress on the use of multiple memory systems: Stress exposure prior to learning shifts the relative use of a hippocampus-dependent declarative or spatial strategy toward a striatal-dependent habit-like strategy (Devan and White, 1999; Kim et al., 2001; Packard and Wingard, 2004). However, more recent evidence indicates that stress and glucocorticoids also directly affect striatal-dependent memory processes (de Quervain et al., 2017; Schwabe and Wolf, 2012). We previously reported that glucocorticoids administered directly into the dorsal striatum enhance the consolidation of memory of inhibitory avoidance and cued water-maze training (Medina et al., 2007; Quirarte et al., 2009). CB1Rs are abundantly expressed within the striatum, as well as in related structures such as the globus pallidus and substantia nigra (Herkenham et al., 1990). However, whether CB1R activity within the dorsal striatum is involved in mediating such glucocorticoid effects on memory consolidation has not been examined.

The present experiments investigated whether CB1R activity within the dorsal striatum modulates memory consolidation of inhibitory avoidance training and whether striatal CB1R activity plays a role in mediating glucocorticoid effects on memory. First, we administered different doses of the cannabinoid agonist WIN55,212-2 into the dorsal striatum immediately after inhibitory avoidance training. Retention of the training was tested 48 h later. Next, we investigated whether a blockade of CB1R activity in the dorsal striatum with posttraining infusions of the antagonist AM251 impairs inhibitory avoidance memory, to determine whether endogenous CB1R activity within this brain region influences consolidation processes. A second series of experiments investigated whether CB1R activity within the dorsal striatum is required for enabling glucocorticoid-induced enhancement of inhibitory avoidance memory. Rats received posttraining infusions of AM251 into the dorsal striatum followed either by an administration of corticosterone or the membrane-impermeable glucocorticoid ligand corticosterone:BSA (CORT:BSA) into the dorsal striatum or by a peripheral administration of corticosterone. Lastly, we investigated whether the interaction between glucocorticoids and the eCB system on inhibitory avoidance memory might be bidirectional in nature. We examined whether a suppression of glucocorticoid signaling by the corticosterone-synthesis inhibitor metyrapone administered before inhibitory avoidance training would block the memory-enhancing effect of WIN55,212-2 administered posttraining into the dorsal striatum.

2. Material and methods

2.1. Animals

Male Wistar rats (n = 262; weighing 250–300 g at the time of surgery), from the breeding colony at the Instituto de Neurobiología, UNAM, were housed individually in a temperature-controlled (22 °C) vivarium room and maintained at a standard 12-h:12-h light:dark cycle (lights on: 7:00–19:00 h) with *ad libitum* access to food and water. Training and testing were performed during the light phase of the cycle between 10:00 and 15:00 h. All procedures were in compliance with NIH guidelines (National Research Council, 2011), NOM-062-ZOO-1999 (NORMA Oficial Mexicana NOM-062-ZOO-1999, 2001NORM, 1999NOM-062-ZOO-1999 (NORMA Oficial Mexicana NOM-062-ZOO-1999, 2001NORM, 1999NOM-062-ZOO-1999 (NORMA Oficial Mexicana NOM-062-ZOO-1999, 2001), and approved by the Ethics Committee of the Instituto de Neurobiología, UNAM.

2.2. Cannula implantation

Rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (Pisabarbital, 50 mg/kg) and given atropine (PiSa, 0.4 mg/kg) to prevent respiratory obstruction. They subsequently received 1 ml of saline intraperitoneally to maintain hydration and facilitate the clearance of drugs. The rats were positioned in a stereotaxic frame (Stoelting Co) and two stainless-steel guide cannulae (11-mm long, 23 gauge) were implanted bilaterally with the cannula tips above the anterior part of the dorsal striatum [anteroposterior: + 0.4 mm to Bregma, mediolateral: + 3.2 mm to midline, dorsoventral: 4.2 mm below skull surface] (Paxinos and Watson, 2007). The cannulae were anchored to the skull with two jeweler's screws and dental acrylic. Stylets (11 mm long) were inserted into each cannula to maintain patency and were removed only during the handling sessions and drug administration. After surgery, the rats were allowed to recover from anesthesia in an incubator until they were fully awake and were then returned to their home cages. They were allowed to recover for 1 week before initiation of behavioral procedures.

2.3. Inhibitory avoidance apparatus and procedures

Rats were trained and tested in a step-through inhibitory avoidance apparatus consisting of two compartments (30 × 30 × 30 cm), separated by a sliding door (Medina et al., 2007). The starting compartment had walls and lid made of red-colored acrylic with a floor of stainless steel bars and was well lit. The shock compartment was a V-shaped alley (20 cm wide at the top and 8 cm wide at the bottom) made of two electrifiable stainless-steel plates and was not illuminated. In the middle of the floor, a 1.5-cm-wide slot separated the two stainless steel plates that made up the walls and floor. The apparatus was located inside a dark, sound-attenuated room provided with background masking noise (San Diego Instruments).

For training, the rat was placed into the starting compartment, facing away from the sliding door, and 10 s later the door was opened. After entering the shock compartment, the door was closed and a single inescapable footshock was delivered using a precision-regulated animal shock generator (Coulbourn Instruments, USA). A low footshock intensity of 0.45 mA for 1 s was used for the cannabinoid and glucocorticoid agonist experiments in order to assess memory enhancement, and a higher footshock intensity of 0.60 mA for 1 s was used for the CB1R antagonist experiment in order to assess memory impairment, thus preventing a possible floor effect. The rat was removed from the apparatus immediately after termination of footshock. Retention was tested 48 h later by placing the rat into the starting compartment and measuring the latency to re-enter the shock compartment with all four paws (maximum latency of 600 s). Longer latencies were interpreted as

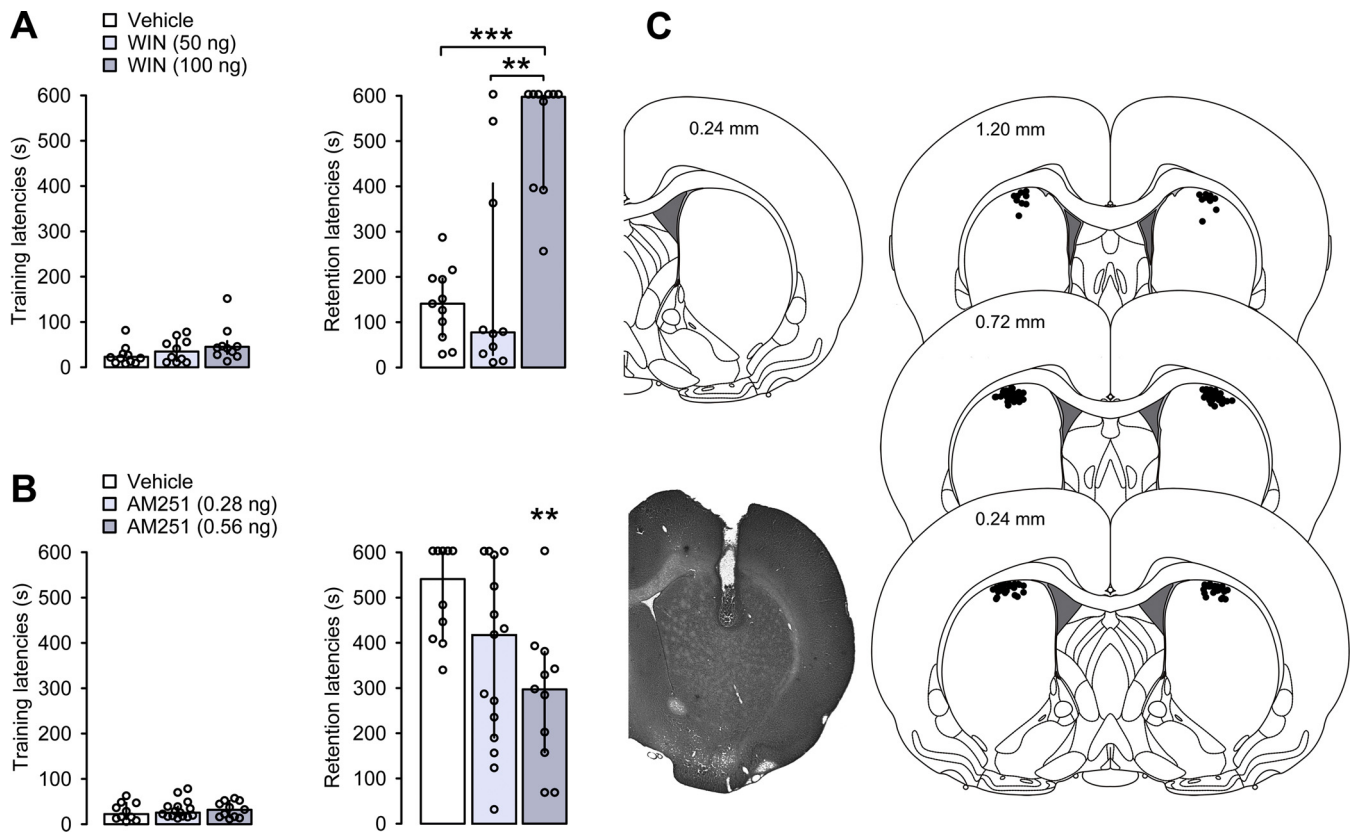

Fig. 1. Effect of cannabinoid and CB1R antagonist administration into the dorsal striatum on memory consolidation of inhibitory avoidance training. **A.** Training and 48-h retention latencies (median and interquartile range) in seconds of rats that received immediate posttraining infusions of the cannabinoid WIN55,212-2 (WIN, 50 or 100 ng) into the dorsal striatum. Dots in the graphs represent individual data points. VEH ($n = 11$), WIN 50 ($n = 10$), WIN 100 ($n = 10$); $^{**}P < .01$, $^{***}P < .001$. **B.** Training and 48-h retention latencies of rats that received immediate posttraining infusions of the CB1R antagonist AM251 (0.28 or 0.56 ng) into the dorsal striatum. Dots in the graphs represent individual data points. VEH ($n = 10$), AM251 0.28 ($n = 11$), AM251 0.56 ($n = 15$); $^{**}P < .01$. **C.** Representative photomicrograph of a cannula track within the dorsal striatum and schematic diagrams illustrating the location of infusion needle tips of 70 randomly selected rats included in the analyses.

indicating better retention. No shock was administered on the retention test trial.

2.4. Local drug administration

The cannabinoid WIN55,212-2 ((R)-(+)-[2,3-dihydro-5-methyl-3[(4-morpholinyl)methyl]pyrrolo[1,2,3-de]-1,4-benzoxazinyl]-(1-naphthalenyl), 50 or 100 ng, Sigma-Aldrich) and CB1R antagonist AM251 (1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-N-1-piperidinyl-1H-pyrazole-3-carboxamide, 0.28 or 0.56 ng, Sigma-Aldrich) were dissolved in a vehicle containing 10% dimethyl sulfoxide in isotonic saline and administered bilaterally into the dorsal striatum immediately after the training trial. For intra-striatal administration of corticosterone (10 or 30 ng, Sigma–Aldrich), the drug was first dissolved in 100% ethanol and subsequently diluted in saline to a final ethanol concentration of 2% (Atsak et al., 2015). The membrane-impermeable glucocorticoid ligand CORT:BSA (corticosterone conjugated to a large albumin molecule, 5 or 10 ng, CUSABIO) was dissolved in a vehicle containing 0.5% ethanol and 0.1% BSA in saline (Atsak et al., 2015; Barsegyan et al., 2010).

Bilateral infusions of drug or vehicle were made by using 30-gauge injection needles connected to 10-µl Hamilton microsyringes by polyethylene tubing. The injection needles protruded 1.0 mm beyond the cannula tips and a 1.0-µl injection volume per hemisphere was infused over a period of 60 s by an automated syringe pump (WPI, model 220i). The injection needles were retained within the cannulae for an additional 60 s to maximize diffusion. If two different drugs had to be administered, these were infused separately: first 0.5 µl of the antagonist followed 10 min later by 0.5 µl of the agonist, always to a total volume of 1.0 µl. Drug solutions were freshly prepared before each experiment.

2.5. Systemic drug administration

Corticosterone (3 mg/kg) was first dissolved in 100% ethanol and subsequently diluted in saline to reach a 5% ethanol concentration (Atsak et al., 2015; Campolongo et al., 2009; Okuda et al., 2004). Drug or vehicle was administered intraperitoneally, in a volume of 1.5 ml/kg, immediately after the posttraining intra-striatal AM251 infusion. Previous findings indicate that this dose induces plasma corticosterone levels that are within the high physiological range (de Quervain et al., 1998). The corticosterone-synthesis inhibitor metyrapone (2-methyl-1,2-di-3-pyridyl-1-propanone, 50 mg/kg, Sigma-Aldrich) was first dissolved in 100% polyethylene glycol, then diluted in saline to a final polyethylene glycol concentration of 40%, and injected intraperitoneally, in a volume of 2 ml/kg, 90 min before training (Sánchez-Resendis et al., 2012). This dose of metyrapone does not completely block corticosterone synthesis but it prevents stress or training-induced increases in circulating corticosterone levels (Roozendaal et al., 1996). Drug solutions were freshly prepared before each experiment.

2.6. Histology

Rats were anesthetized with an overdose of sodium pentobarbital (100 mg/kg, ip) and perfused transcardially with 0.9% saline followed by 4% formaldehyde. The brains were collected and kept in 4%

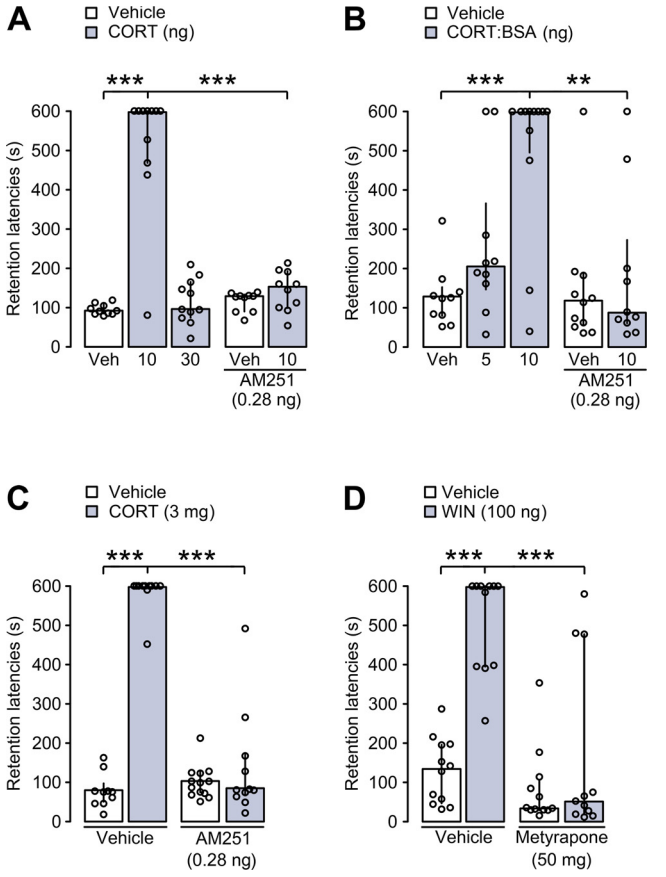

Fig. 2. Interacting effects of glucocorticoids and the eCB activity in the dorsal striatum in regulating consolidation of inhibitory avoidance memory. **A.** Forty-eight-hour retention latencies (median and interquartile range) in seconds of rats that received posttraining infusions of the CB1R antagonist AM251 (0.28 ng) and corticosterone (CORT, 10 or 30 ng) into the dorsal striatum. VEH-VEH ($n = 10$), VEH-CORT10 ($n = 10$), VEH-CORT30 ($n = 11$), AM251-VEH ($n = 10$), AM251-CORT10 ($n = 10$); ***$P < .001$. **B.** Forty-eight-hour retention latencies of rats that received posttraining infusions of AM251 (0.28 ng) and CORT:BSA (5 or 10 ng) into the dorsal striatum. VEH-VEH ($n = 10$), VEH-CORT:BSA5 ($n = 10$), VEH-CORT:BSA10 ($n = 12$), AM251-VEH ($n = 11$), AM251-CORT:BSA10 ($n = 10$); **$P < .01$, ***$P < .001$. **C.** Forty-eight-hour retention latencies of rats that received posttraining infusions of AM251 (0.28 ng) into the dorsal striatum together with a systemic injection of CORT (3 mg/kg). VEH-VEH ($n = 10$), VEH-CORT ($n = 11$), AM251-VEH ($n = 13$), AM251-CORT ($n = 11$); ***$P < .001$. **D.** Forty-eight-hour retention latencies of rats given a systemic injection of metyrapone (MET, 50 mg/kg) 90 min before training and immediate posttraining infusions of the cannabinoid WIN55,212-2 (WIN, 100 ng) into the dorsal striatum. VEH-VEH ($n = 12$), VEH-WIN ($n = 11$), MET-VEH ($n = 12$), MET-WIN ($n = 11$); ***$P < .001$. Dots in the different graphs represent individual data points.

formaldehyde. Fifty-micrometer-thick coronal sections were cut on a microtome, mounted on gelatin-coated slides, and stained with the Nissl technique. The location of injection needle tips was examined under a light microscope by an observer blind to the treatment condition. The injection needles of 5% of the rats were located outside the dorsal striatum and were discarded from statistical analysis.

2.7. Statistical analysis

Inhibitory avoidance training and retention latencies were analyzed with non-parametric Kruskal-Wallis tests, when appropriate followed by Mann-Whitney's U tests. Additionally, a difference score between retention and training latencies was calculated and analyzed with Kruskal-Wallis and Mann-Whitney's U tests. For all tests, $P < .05$ was accepted as statistical significance. The number of animals per group is indicated in the figure legends.

3. Results

3.1. Effect of intra-striatal cannabinoid and CB1R antagonist administration on inhibitory avoidance memory

We investigated whether the cannabinoid agonist WIN55,212-2 infused into the dorsal striatum immediately after inhibitory avoidance training, with a mild footshock intensity of 0.45 mA, enhances memory consolidation of the training experience. As shown in Fig. 1A, step-through latencies during training, before footshock and drug treatment, did not differ between groups ($H_{(2)} = 4.88$, $P = .09$). Forty-eight-hour retention latencies of rats administered vehicle into the dorsal striatum were significantly longer than their entrance latencies during the training trial ($P = .001$), indicating that the rats retained memory of the shock experience. WIN55,212-2 (50 or 100 ng) induced dose-dependent retention enhancement ($H_{(2)} = 15.67$, $P < .001$). Mann-Whitney *post-hoc* comparison tests indicated that retention latencies of rats given the higher dose of WIN55,212-2 (100 ng) were significantly longer than those of rats administered either vehicle ($P < .001$) or the lower dose of WIN55,212-2 (50 ng; $P < .01$). The difference score (the difference between retention and training latencies) of animals given the higher dose of WIN55,212-2 was also significantly larger than that of animals administered vehicle ($P < .05$) or the lower dose of WIN55,212-2 ($P < .05$) (Supplementary material Fig. 1A).

Next, we examined whether posttraining blockade of CB1R activity in the dorsal striatum with AM251 impairs the consolidation of inhibitory avoidance memory. For this, rats were trained with a higher shock intensity (0.60 mA) which resulted in longer retention latencies in vehicle-treated control rats. As shown in Fig. 1B, training latencies did not differ between posttraining drug treatment groups ($H_{(2)} = 0.86$, $P = .65$). AM251 (0.28 or 0.56 ng) dose-dependently impaired 48-h retention performance ($H_{(2)} = 8.89$, $P = .01$). *Post-hoc* comparison tests indicated that rats treated with the higher dose of AM251 (0.56 ng) had significantly shorter retention latencies compared to rats administered vehicle ($P < .01$). The lower dose of AM251 (0.28 ng) did not significantly impair retention. The difference score of animals administered either the higher or lower dose of AM251 was significantly different from that of animals given vehicle ($P < .05$) (Supplementary material Fig. 1B). Together, these findings indicate that striatal CB1Rs are involved in modulating the consolidation of memory of inhibitory avoidance training.

Fig. 1C illustrates the infusion needle sites within the dorsal striatum of 70 randomly selected animals used in this study.

3.2. Interacting effects of glucocorticoids and CB1R activity in the dorsal striatum on inhibitory avoidance memory

We investigated whether CB1R activity within the dorsal striatum is required for enabling glucocorticoid effects on memory consolidation. First, AM251 (0.28 ng) and corticosterone (10 or 30 ng) were administered both into the dorsal striatum immediately after inhibitory avoidance training with a low shock intensity (0.45 mA). Training latencies did not differ between posttraining drug treatment groups ($H_{(4)} = 3.34$, $P = .50$) (Supplementary material Fig. 2A). As shown in Fig. 2A, Kruskal-Wallis test for 48-h retention latencies indicated a significant treatment effect ($H_{(4)} = 22.35$, $P < .001$). The lower (10 ng), but not higher (30 ng), dose of corticosterone increased retention latencies ($P < .001$). Most importantly, this low dose of AM251 did not impair retention *per se* but blocked the memory-enhancing effect of corticosterone. Retention latencies of rats treated with corticosterone (10 ng) together with AM251 were significantly shorter than those of rats administered corticosterone alone ($P < .001$). The difference score of rats treated with vehicle was also significantly different

from that of rats administered the lower dose of corticosterone ($P < .001$) (Supplementary material Fig. 3A). Moreover, the difference score of rats given the lower dose of corticosterone differed significantly from that of rats administered the higher dose of corticosterone, AM251-vehicle or AM251-corticosterone (all, $P < .001$) (Supplementary material Fig. 3A).

To investigate whether such glucocorticoid-eCB interactions involve the activation of a corticosteroid receptor on the cell surface (Atsak et al., 2015; Hill and McEwen, 2009), rats received posttraining infusions of the membrane-impermeable glucocorticoid ligand CORT:BSA (5 or 10 ng) into the dorsal striatum, either alone or together with AM251 (0.28 ng). Training latencies did not differ between posttraining drug treatment groups ($H_{(4)} = 4.38$, $P = .36$) (Supplementary material Fig. 2B). As shown in Fig. 2B, Kruskal-Wallis test for 48-h retention latencies revealed a significant treatment effect ($H_{(4)} = 16.72$, $P = .002$). *Post-hoc* analysis indicated that both doses of CORT:BSA induced longer retention latencies (5 ng: $P < .05$; 10 ng: $P < .001$) and that AM251 prevented the CORT:BSA effect ($P < .01$). The difference score of animals given 10 ng of CORT:BSA was also significantly different from that of all other treatment groups ($P < .05$) (Supplementary material Fig. 3B).

We further investigated whether posttraining blockade of striatal CB1Rs would be sufficient to also prevent the memory-enhancing effect of systemically administered corticosterone. Training latencies did not differ between posttraining drug treatment groups ($H_{(3)} = 1.15$, $P = .77$) (Supplementary material Fig. 2C). As shown in Fig. 2C, Kruskall-Wallis test for 48-h retention latencies indicated a significant treatment effect ($H_{(3)} = 25.57$, $P < .001$). Systemic corticosterone (3 mg/kg) administration induced longer retention latencies ($P < .001$) whereas co-administration of AM251 (0.28 ng) into the dorsal striatum blocked the systemic corticosterone effect ($P < .001$). Additionally, the difference score of the corticosterone group differed significantly from that of all other treatment groups ($P < .001$) (Supplementary material Fig. 3C).

These findings thus clearly indicate that glucocorticoid effects on inhibitory avoidance memory require concurrent CB1R activity within the dorsal striatum. To investigate whether the reverse is also true, i.e., striatal cannabinoid effects on memory require glucocorticoid signaling, the corticosterone-synthesis inhibitor metyrapone was injected intraperitoneally 90 min before training and a memory-enhancing dose of WIN55,212-2 (100 ng) was administered into the dorsal striatum immediately after inhibitory avoidance training. Metyrapone pretreatment did not significantly affect entrance latencies during the training trial ($H_{(3)} = 7.64$, $P = .054$) (Supplementary material Fig. 2D). As shown in Fig. 2D, Kruskal-Wallis test for 48-h retention latencies indicated a significant treatment effect ($H_{(3)} = 22.92$, $P < .001$). WIN55,212-2 (100 ng) treatment increased retention latencies ($P < .001$) and metyrapone pretreatment blocked this WIN55,212-2 effect ($P < .001$). The difference score of animals administered vehicle systemically and WIN55,212-2 into the dorsal striatum differed significantly from that of the vehicle-vehicle and metyrapone-WIN55,212-2 treatment groups ($P < .001$) (Supplementary material Fig. 3D). These findings thus suggest the existence of a bidirectional interaction between the glucocorticoid and eCB systems within the dorsal striatum in modulating inhibitory avoidance memory.

4. Discussion

The present findings provide evidence that CB1R activity within the dorsal striatum is involved in modulating the consolidation of inhibitory avoidance memory and that striatal CB1R activity is essential for mediating glucocorticoid effects on inhibitory avoidance memory.

We show that posttraining bilateral infusions of the cannabinoid WIN55,212-2 administered into the dorsal striatum induced dose-dependent enhancement of inhibitory avoidance memory; in contrast, bilateral posttraining blockade of CB1R activity within the dorsal striatum with AM251 impaired retention. These findings indicate that the eCB system in the dorsal striatum is involved in the regulation of memory consolidation. Goodman and Packard (2014) reported that systemic or intra-striatal administration of WIN55,212-2 impaired memory consolidation of dorsal striatal-dependent cued water-maze training. Conflicting findings with respect to the direction of cannabinoid effects on memory have also been reported in other brain regions. For example, some studies reported that cannabinoid agonists administered into the BLA or hippocampus impair the encoding or consolidation of memory of spatial water-maze, inhibitory avoidance and object recognition training (Barros et al., 2004), whereas we have previously shown that WIN55,212-2 administered into the BLA immediately after inhibitory avoidance training enhances the consolidation of long-term memory, while inhibition of endogenous cannabinoid signaling within the BLA with posttraining infusions of the CB1R antagonist AM251 impairs inhibitory avoidance memory (Campolongo et al., 2009; Morena et al., 2014). Differences in arousal level associated with the learning task, drug dose and administration regimen (e.g. pretraining vs posttraining administration) as well as retention performance of control animals could all have contributed to these opposite findings (Campolongo et al., 2013).

The dorsal striatum can be divided into several functionally distinct subregions (Goodman and Packard, 2017; Voorn et al., 2004; Yin and Knowlton, 2004). The dorsolateral region of the striatum is engaged in processing procedural memory, whereas the dorsomedial region is primarily involved in spatial-contextual processing (Devan and White, 1999; Liljeholm and O'Doherty, 2012; Voorn et al., 2004; Yin et al., 2009). In the present study, we did not differentiate between the dorsolateral and dorsomedial regions. As CB1Rs are highly expressed throughout the dorsolateral and dorsomedial striatum (Herkenham et al., 1990), our drug manipulations might have affected CB1R activity within the entire dorsal area of the striatum. The acquisition of inhibitory avoidance training requires the encoding of both contextual-spatial and procedural aspects of information (Medina et al., 2007). Therefore, it is possible that changes in retention latencies induced by the cannabinoid or CB1R antagonist manipulations reflect alterations in the strength of both contextual-spatial and procedural aspects of inhibitory avoidance memory. Future studies should investigate the specific role of the eCB system within the dorsolateral and dorsomedial regions of the striatum in influencing the consolidation of contextual-spatial and habitual-like procedural types of memory. Some studies have examined the effect of a selective cannabinoid manipulation within the dorsolateral striatum on habitual-like memory. It has been reported that the administration of a high dose of WIN55,212-2 into the dorsolateral striatum impaired habitual-like memory on an aversive cued water-maze task (Goodman and Packard, 2014). Interestingly, administration of the CB1R antagonist AM251 into the dorsolateral striatum induced a comparable impairment of habitual-like memory in an appetitive task (Rueda-Orozco et al., 2008). Some findings suggest that acute cannabinoid manipulations of the dorsolateral striatum, regardless of the agonistic or antagonistic action, might have a detrimental effect on habitual-like behavior, whereas sustained, chronic manipulations favor the prevalence of non-cognitive flexible habitual memory (Goodman and Packard, 2015). Findings from human cannabis consumers with a history of prolonged usage also indicate a tendency to more predominantly utilize a habitual-like behavioral strategy (Goodman and Packard, 2015).

Endogenously released eCB ligands function as diffusible and short-lived modulators that may transmit signals retrogradely from post-synaptic to presynaptic neurons (Lovinger and Mathur, 2017; Ohno-Shosaku and Kano, 2014). Activation of CB1Rs decreases neurotransmitter release (Ohno-Shosaku and Kano, 2014) via a rapid inhibition of Ca^{2+} entry into the terminals. We previously reported that inhibitory avoidance training induces a rapid elevation of anandamide, but not 2-arachydonoylglycerol, levels within the BLA, hippocampus and prefrontal cortex, and that pharmacological augmentation of

anandamide levels in these brain regions enhances inhibitory avoidance memory through CB1Rs (Morena et al., 2014). However, it is currently not known whether anandamide is also the main eCB ligand regulating striatal cannabinoid effects on memory consolidaton. In the striatum, CB1Rs are abundantly expressed on medium spiny neurons (Herkenham et al., 1990), and modulate plasticity through eCB-dependent long-term depression (eLTD). The striatum receives a vast amount of projections from motor cortex but also from associative and frontal cortices, thus this structure computes multimodal inputs and integrates diverse types of information (Goodman and Packard, 2014). Interestingly, eLTD can influence both glutamatergic and GABAergic activity, modulating excitatory inputs from associative cortices and inhibitory outputs to striato-nigral or striato-pallidal circuits (Lovinger and Mathur, 2017).

Our findings clearly demonstrate that CB1R activity within the dorsal striatum is essentially involved in regulating glucocorticoid effects on memory consolidation. These findings are highly comparable to those of prior studies investigating glucocorticoid-eCB interactions within the BLA or hippocampus on memory (Atsak et al., 2015; Campolongo et al., 2009; Morena et al., 2014). Previously, we have shown that corticosterone administration into the dorsal striatum enhances inhibitory avoidance memory (Medina et al., 2007) as well as memory of more typical dorsal striatum-dependent procedural and habit-like forms of training (Quirarte et al., 2009; Siller-Pérez et al., 2017). Droste et al. (2008) reported that forced swimming stress induces a marked increase in free corticosterone levels within the dorsal striatum, indicating that the striatum is sensitive and responsive to a physiological stressor. Inhibitory avoidance training is known to increase corticosterone plasma levels (González-Franco et al., 2017), which subsequently might give rise to elevated eCB levels (Morena et al., 2014). Glucocorticoids, via a corticosteroid receptor on the cell surface, rapidly increase eCB release in different brain regions (Hill et al., 2010a). Such findings suggest that glucocorticoids might enhance memory consolidation, at least in part, by stimulating downstream eCB neurotransmission (Atsak et al., 2015; Campolongo et al., 2009; Morena et al., 2014). Our finding that CORT:BSA which does not readily cross the cell membrane and thus likely activates a corticosteroid receptor on or near the cell surface also enhances inhibitory avoidance memory in a CB1R-dependent fashion is consistent with this view (Atsak et al., 2015). However, our finding that corticosterone-synthesis inhibition also impaired the memory enhancement induced by striatal administration of the cannabinoid WIN55,212-2 supports a bidirectional relationship between glucocorticoids and the eCB system. This observation is in accordance with the evidence that systemic administration of WIN55,212-2 regulates adrenocortical activity, which in turn contributes to the environmentally sensitive effects of systemically administered cannabinoids on short- and long-term retention of object recognition memory (Campolongo et al., 2013).

5. Conclusions

Our findings provide evidence that the eCB system, in close interaction with glucocorticoids, is involved in modulating plasticity changes underlying memory consolidation not only in highly stress-sensitive corticolimbic structures but also within the dorsal striatum.

Declarations of interest

None.

Author contributions

CSP, BR, PC and GQ designed the experiments; CSP, AFI and ESB performed the experiments; CSP and NS performed the statistical analyses; CSP, NS, RAPA, PC, BR and GQ wrote the paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.psyneuen.2018.08.021.

References

Atsak, P., Hauer, D., Campolongo, P., Schelling, G., Fornari, R.V., Roozendaal, B., 2015. Endocannabinoid signaling within the basolateral amygdala integrates multiple stress hormone effects on memory consolidation. Neuropsychopharmacology 40, 1485–1494.
Balsevich, G., Petrie, G.N., Hill, M.N., 2017. Endocannabinoids: effectors of glucocorticoid signaling. Front. Neuroendocrinol. 47, 86–108.
Barros, D.M., Carlis, V., Maidana, M., Silva, E.S., Baisch, A.L.M., Ramirez, M.R., Izquierdo, I., 2004. Interactions between anandamide-induced anterograde amnesia and post-training memory modulatory systems. Brain Res. 1016, 66–71.
Barsegyan, A., Mackenzie, S.M., Kurose, B.D., McGaugh, J.L., Roozendaal, B., 2010. Glucocorticoids in the prefrontal cortex enhance memory consolidation and impair working memory by a common neural mechanism. Proc. Natl. Acad. Sci. U. S. A. 107, 16655–16660.
Buchanan, T.W., Lovallo, W.R., 2001. Enhanced memory for emotional material following stress-level cortisol treatment in humans. Psychoneuroendocrinology 26, 307–317.
Campolongo, P., Roozendaal, B., Trezza, V., Hauer, D., Schelling, G., McGaugh, J.L., Cuomo, V., 2009. Endocannabinoids in the rat basolateral amygdala enhance memory consolidation and enable glucocorticoid modulation of memory. Proc. Natl. Acad. Sci. U. S. A. 106, 4888–4893.
Campolongo, P., Morena, M., Scaccianoce, S., Trezza, V., Chiarotti, F., Schelling, G., Cuomo, V., Roozendaal, B., 2013. Novelty-induced emotional arousal modulates cannabinoid effects on recognition memory and adrenocortical activity. Neuropsychopharmacology 38, 1276–1286.
de Kloet, E.R., Oitzl, M.S., Joëls, M., 1999. Stress and cognition: are corticosteroids good or bad guys? Trends Neurosci. 22, 422–426.
de Quervain, D.J., Roozendaal, B., McGaugh, J.L., 1998. Stress and glucocorticoids impair retrieval of long-term spatial memory. Nature 394, 787–790.
de Quervain, D., Schwabe, L., Roozendaal, B., 2017. Stress, glucocorticoids and memory: implications for treating fear-related disorders. Nat. Rev. Neurosci. 18, 7-19.
Devan, B.D., White, N.M., 1999. Parallel information processing in the dorsal striatum: relation to hippocampal function. J. Neurosci. 19, 2789–2798.
Di, S., Tasker, J.G., 2008. Rapid synapse-specific regulation of hypothalamic magnocellular neurons by glucocorticoids. Prog. Brain Res. 170, 379–388.
Di, S., Malcher-Lopes, R., Halmos, K.C., Tasker, J.G., 2003. Nongenomic glucocorticoid inhibition via endocannabinoid release in the hypothalamus: a fast feedback mechanism. J. Neurosci. 23, 4850–4857.
Di Marzo, V., Melck, D., Bisogno, T., De Petrocellis, L., 1998. Endocannabinoids: endogenous cannabinoid receptor ligands with neuromodulatory action. Trends Neurosci. 21, 521–528.
Droste, S.K., de Groote, L., Atkinson, H.C., Lightman, S.L., Reul, J.M.H.M., Linthorst, A.C.E., 2008. Corticosterone levels in the brain show a distinct ultradian rhythm but a delayed response to forced swim stress. Endocrinology 149, 3244–3253.
González-Franco, D.A., Ramírez-Amaya, V., Joseph-Bravo, P., Prado-Alcalá, R.A., Quirarte, G.L., 2017. Differential Arc protein expression in dorsal and ventral striatum after moderate and intense inhibitory avoidance training. Neurobiol. Learn. Mem. 140, 17–26.
Goodman, J., Packard, M.G., 2014. Peripheral and intra-dorsolateral striatum injections of the cannabinoid receptor agonist WIN 55,212-2 impair consolidation of stimulus-response memory. Neuroscience 274, 128–137.
Goodman, J., Packard, M.G., 2015. The influence of cannabinoids on learning and memory processes of the dorsal striatum. Neurobiol. Learn. Mem. 125, 1–14.
Goodman, J., Packard, M.G., 2017. Memory systems of the basal ganglia. In: Steiner, H., Tseng, K.Y. (Eds.), Handbook of Basal Ganglia Structure and Function. Elsevier, pp. 725–740.
Goodman, J., Leong, K.-C., Packard, M.G., 2012. Emotional modulation of multiple memory systems: implications for the neurobiology of post-traumatic stress disorder. Rev. Neurosci. 23, 627.
Gray, J.M., Vecchiarelli, H.A., Hill, M.N., 2014. Endocannabinoid signaling and synaptic plasticity during stress. In: Popoli, M., Diamond, D., Sanacora, G. (Eds.), Synaptic Stress and Pathogenesis of Neuropsychiatric Disorders. Springer, New York, pp. 99–124.
Herkenham, M., Lynn, A.B., Little, M.D., Johnson, M.R., Melvin, L.S., de Costa, B.R., Rice, K.C., 1990. Cannabinoid receptor localization in brain. Proc. Natl. Acad. Sci. U. S. A.

87, 1932–1936.
Hill, M.N., McEwen, B.S., 2009. Endocannabinoids: the silent partner of glucocorticoids in the synapse. Proc. Natl. Acad. Sci. U. S. A. 106, 4579–4580.
Hill, M.N., Karatsoreos, I.N., Hillard, C.J., McEwen, B.S., 2010a. Rapid elevations in limbic endocannabinoid content by glucocorticoid hormones in vivo. Psychoneuroendocrinology 35, 1333–1338.
Hill, M.N., McLaughlin, R.J., Bingham, B., Shrestha, L., Lee, T.T.Y., Gray, J.M., Hillard, C.J., Gorzalka, B.B., Viau, V., 2010b. Endogenous cannabinoid signaling is essential for stress adaptation. Proc. Natl. Acad. Sci. U. S. A. 107, 9406–9411.
Hill, M.N., McLaughlin, R.J., Pan, B., Fitzgerald, M.L., Roberts, C.J., Lee, T.T., Karatsoreos, I.N., Mackie, K., Viau, V., Pickel, V.M., McEwen, B.S., Liu, Q.S., Gorzalka, B.B., Hillard, C.J., 2011. Recruitment of prefrontal cortical endocannabinoid signaling by glucocorticoids contributes to termination of the stress response. J. Neurosci. 31, 10506–10515.
Hillard, C.J., Beatka, M., Sarvaideo, J., 2016. Endocannabinoid signaling and the hypothalamic-pituitary-adrenal axis. Compr. Physiol. 7, 1–15.
Kim, J.J., Lee, H.J.J., Han, J.S., Packard, M.G., 2001. Amygdala is critical for stress-induced modulation of hippocampal long-term potentiation and learning. J. Neurosci. 21, 5222–5228.
Kim, J.J., Song, E.Y., Kim, J.J., Song, E.Y., Kosten, T.A., 2006. Stress effects in the hippocampus: synaptic plasticity and memory. Stress 9, 1–11.
Kuhlmann, S., Wolf, O.T., 2006. Arousal and cortisol interact in modulating memory consolidation in healthy young men. Behav. Neurosci. 120, 217–223.
Liljeholm, M., O'Doherty, J.P., 2012. Contributions of the striatum to learning, motivation, and performance: an associative account. Trends Cogn. Sci. 16, 467–475.
Lovinger, D.M., Mathur, B.N., 2017. Endocannabinoid signaling in the striatum. In: Steiner, H., Tseng, K.Y. (Eds.), Handbook of Basal Ganglia Structure and Function. Elsevier, pp. 197–215.
Lupien, S.J., McEwen, B.S., 1997. The acute effects of corticosteroids on cognition: integration of animal and human model studies. Brain Res. Rev. 24, 1–27.
Lutz, B., Marsicano, G., Maldonado, R., Hillard, C.J., 2015. The endocannabinoid system in guarding against fear, anxiety and stress. Nat. Rev. Neurosci. 16, 705.
McEwen, B.S., Sapolsky, R.M., 1995. Stress and cognitive function. Curr. Opin. Neurobiol. 5, 205–216.
McGaugh, J.L., Roozendaal, B., 2002. Role of adrenal stress hormones in forming lasting memories in the brain. Curr. Opin. Neurobiol. 12, 205–210.
Medina, A.C., Charles, J.R., Espinoza-González, V., Sánchez-Resendis, O., Prado-Alcalá, R.A., Roozendaal, B., Quirarte, G.L., 2007. Glucocorticoid administration into the dorsal striatum facilitates memory consolidation of inhibitory avoidance training but not of the context or footshock components. Learn. Mem. 14, 673–677.
Morena, M., Roozendaal, B., Trezza, V., Ratano, P., Peloso, A., Hauer, D., Atsak, P., Trabace, L., Cuomo, V., McGaugh, J.L., Schelling, G., Campolongo, P., 2014. Endogenous cannabinoid release within prefrontal-limbic pathways affects memory consolidation of emotional training. Proc. Natl. Acad. Sci. U. S. A. 111, 18333–18338.
Morena, M., Leitl, K.D., Vecchiarelli, H.A., Gray, J.M., Campolongo, P., Hill, M.N., 2016. Emotional arousal state influences the ability of amygdalar endocannabinoid signaling to modulate anxiety. Neuropharmacology 111, 59–69.
National Research Council, 2011. Guide for the Care and Use of Laboratory Animals, 8th ed. National Academy of Sciences, Washington DC.
NORMA Oficial Mexicana NOM-062-ZOO-1999, 2001. Especificaciones técnicas para la producción, cuidado y uso de los animales de laboratorio. SENASICA, México, pp. 65.
Ohno-Shosaku, T., Kano, M., 2014. Endocannabinoid-mediated retrograde modulation of synaptic transmission. Curr. Opin. Neurobiol. 29, 1–8.
Okuda, S., Roozendaal, B., McGaugh, J.L., 2004. Glucocorticoid effects on object recognition memory require training-associated emotional arousal. Proc. Natl. Acad. Sci. U. S. A. 101, 853–858.
Packard, M.G., Wingard, J.C., 2004. Amygdala and "emotional" modulation of the relative use of multiple memory systems. Neurobiol. Learn. Mem. 82, 243–252.
Paxinos, G., Watson, C., 2007. The Rat Brain in Stereotaxic Coordinates, 6th ed. Academic Press, Burlington, MA.
Quirarte, G.L., de la Teja, I.S., Casillas, M., Serafín, N., Prado-Alcalá, R.A., Roozendaal, B., 2009. Corticosterone infused into the dorsal striatum selectively enhances memory consolidation of cued water-maze training. Learn. Mem. 16, 586–589.
Rey, A.A., Purrio, M., Viveros, M.P., Lutz, B., 2012. Biphasic effects of cannabinoids in anxiety responses: CB1 and GABA(B) receptors in the balance of GABAergic and glutamatergic neurotransmission. Neuropsychopharmacology 37, 2624–2634.
Roozendaal, B., McGaugh, J.L., 2011. Memory modulation. Behav. Neurosci. 125, 797–824.
Roozendaal, B., Bohus, B., McGaugh, J.L., 1996. Dose-dependent suppression of adrenocortical activity with metyrapone: effects on emotion and memory. Psychoneuroendocrinology 21, 681–693.
Roozendaal, B., McEwen, B.S., Chattarji, S., 2009. Stress, memory and the amygdala. Nat. Rev. Neurosci. 10, 423–433.
Rueda-Orozco, P.E., Montes-Rodriguez, C.J., Soria-Gomez, E., Méndez-Díaz, M., Prospéro-García, O., 2008. Impairment of endocannabinoids activity in the dorsolateral striatum delays extinction of behavior in a procedural memory task in rats. Neuropharmacology 55, 55–62.
Sánchez-Resendis, O., Medina, A.C., Serafín, N., Prado-Alcalá, R.A., Roozendaal, B., Quirarte, G.L., 2012. Glucocorticoid-cholinergic interactions in the dorsal striatum in memory consolidation of inhibitory avoidance training. Front. Behav. Neurosci. 6, 33.
Sandi, C., Pinelo-Nava, M.T., 2007. Stress and memory: behavioral effects and neurobiological mechanisms. Neural Plast. 2007, 1–20.
Schwabe, L., Wolf, O.T., 2012. Stress modulates the sengagement of multiple memory systems in classification learning. J. Neurosci. 32, 11042–11049.
Schwabe, L., Wolf, O.T., 2013. Stress and multiple memory systems: from' thinking' to' doing'. Trends Cogn. Sci. 17, 68.
Siller-Pérez, C., Serafín, N., Prado-Alcalá, R.A., Roozendaal, B., Quirarte, G.L., 2017. Glucocorticoid administration into the dorsolateral but not dorsomedial striatum accelerates the shift from a spatial toward procedural memory. Neurobiol. Learn. Mem. 141, 124–133.
Voorn, P., Vanderschuren, L., Groenewegen, H.J., Robbins, T.W., Pennartz, C.M.A., 2004. Putting a spin on the dorsal-ventral divide of the striatum. Trends Neurosci. 27, 468–474.
Yin, H.H., Knowlton, B.J., 2004. Contributions of striatal subregions to place and response learning. Learn. Mem. 11, 459–463.
Yin, H.H., Mulcare, S.P., Hilario, M.R.F., Clouse, E., Holloway, T., Davis, M.I., Hansson, A.C., Lovinger, D.M., Costa, R.M., 2009. Dynamic reorganization of striatal circuits during the acquisition and consolidation of a skill. Nat. Neurosci. 12, 333–341.