

Chapter 17

Cannabinoid-Dopamine Interactions: Modulation of Midbrain DA Neurons by Endocannabinoids

François Georges and Miriam Melis

Abstract A key feature of human and animal behavior is to learn from environmental stimuli to adapt efficiently. Under physiological conditions, dopaminergic (DA) neurons are used to evaluate and learn new sensory information and adjust behavior to maximize reward and minimize aversive consequences. The two main DA pathways in the mesencephalon originate from the substantia nigra pars compacta (SNpc) and the ventral tegmental area (VTA). Both *in vivo* and *in vitro* studies have established that DA neurons exhibit spontaneous spike firing that is driven by intrinsic electrophysiological properties, with their activity modulated by afferent inputs and a number of neuromodulators, including endocannabinoids. In the VTA and SNpc, cannabinoid type 1-(CB₁) and ionotropic transient receptor potential vanilloid type 1 (TRPV1) receptors are abundantly expressed as well as their endogenous ligands, mainly anandamide and 2-arachidonoylglycerol. This chapter attempts to summarize some of the major research findings demonstrating that SNpc and VTA DA neurons vary significantly in their molecular and physiological properties according to target location, and that endocannabinoids act on GABAergic, glutamatergic and cholinergic terminals to participate in discrete mechanisms aimed at DA cell homeostatic regulation. As a result, given the role of the endocannabinoid system in modulating DA neuronal function of the SNpc and the VTA, they might take part in associative learning, reward signaling, goal directed behavior, motor skill learning and action-habit transformation. These considerations help explaining the correlation between an unbalanced endocannabinoid signal and altered DA-dependent processes underpinning diverse pathological conditions of both nigrostriatal and mesocorticolimbic systems.

Keywords Dopamine neurons · Ventral tegmental area · Substantia nigra pars compacta · Endocannabinoids · Peroxisome-proliferator-activated receptors · Reward · Rodent · Synaptic plasticity · Vanilloid receptors

F. Georges (✉)
IINS-UMR5297, CNRS, Bordeaux, France
e-mail: francois.georges@u-bordeaux.fr

M. Melis
Department of Biomedical Sciences, University of Cagliari, Cagliari, Italy

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Introduction

The brain constantly receives and evaluates sensory information, and adjusts behavioral output in a flexible fashion in order to maximize reward, and minimize aversive consequences. Midbrain dopamine (DA) pathways control key physiological functions related to locomotor activity, short-term and working memory, associative learning, attention, and novelty encoding. Consequently, abnormal DA system function has long been implicated in both neurological and psychiatric disorders. The endocannabinoid system controls most of the above cited physiological functions, and participates in modulation of neuronal excitability and various forms of synaptic plasticity of midbrain DA neurons [1, 2].

In the present chapter, we will focus on the two main DA pathways originating in the mesencephalon: the substantia nigra pars compacta (SNpc) and the ventral tegmental area (VTA), whose modulation by endogenous cannabinoids has been extensively reviewed [2–5].

The endogenous system comprises a metabolic apparatus to produce, release and eventually transport the endogenous ligands (i.e. endocannabinoids), and their receptors (i.e. cannabinoid receptors, CB). In the VTA and SNpc, cannabinoid type 1-(CB₁) receptors are abundantly expressed [6–8] as well as their endogenous ligands, mainly anandamide and 2-arachidonoylglycerol (2-AG) [9, 10]. The canonical mechanism of action proposed for endocannabinoids posits an *on demand* release of these lipid molecules by the postsynaptic neuron [11], which retrogradely travel across the synaptic cleft to bind to and activate CB₁ receptors located on the presynaptic terminals [12, 13]. The result of such an activation is a reduction of neurotransmitter release in a short- or a long-term manner [12, 14]. Through this mechanism of action, endocannabinoid signaling contributes to different forms of short- and long-term synaptic plasticity at both excitatory and inhibitory synapses [12, 15, 16].

Long-term forms of synaptic plasticity are widely considered indicative of long lasting adaptations of individual synapses, circuits or neural networks, underlying changes in behavior. By mediating and/or regulating long-term forms of synaptic plasticity, endocannabinoids can, therefore, influence the repertoire of enduring modifications of individual synapses, circuits or neural networks and the consequent behavior [15].

Short-term forms of synaptic plasticity, which are rapid means for a bidirectional and reversible modulation of synaptic strength, serve as important mechanisms to modify synaptic functions during computation [17]. Particularly, short-term forms of synaptic plasticity are often viewed as dynamic filters used for transmission of specific input frequencies or patterns, and are key in mechanisms regulating a proper scaling of synaptic inputs [17–21]. By modulating this dynamic gain control mechanism, endocannabinoids can guarantee a proper accuracy of input information [22]. These general rules apply to the VTA [2, 3] as well to the SNpc [23, 24].

Early work demonstrated that DA neurons located in the SNpc and the VTA provide a prediction-error signal for reward-mediated learning [25, 26], and play a central role in encoding positive and negative motivational signals [27, 28]. In strik-

ing contrast, negative outcomes are supposed to generate a negative (inhibitory) prediction-error signal in the same neuronal population. Although DA neurons exhibit considerable heterogeneity regarding their inputs, their projection targets and their basic pharmacological properties [29–31], it was widely assumed that they exhibit homogeneous appetitive and aversive coding across the entire population. The interpretation of these results led researchers to the oversimplified assumption that midbrain DA neurons may operate as a single functional unit. However, this hypothesis is not consistent with recent findings [32]. Particularly, it has been reported that a sub-population of DA neurons exhibit excitatory responses to novel aversive sensory stimuli [27, 33], and maintain this excitatory pattern as the animals learn about negative outcome associated with these aversive stimuli [27]. In addition, aversive stimuli evoke DA release at projection targets [34–36]. Finally, yet importantly, DA plays a critical role during learning of aversive association in both immature and mature brain [37, 38].

As any group of neurons defined by their location and neurotransmitter content, DA cell function is determined by the circuit in which they are embedded, and the behavioral output for this latter. The notion that sub-populations of midbrain DA neurons are integrated in distinct neuronal circuits is also substantiated by studies showing different patterns of DA release in discrete VTA projection targets. As an example, infusion of the AMPA/kainate receptor antagonist LY293558 into the VTA increases the DA levels in the nucleus accumbens (NAc), but decreases them in the prefrontal cortex (PFC) [39]. Furthermore, Lammel and colleagues [40, 41] demonstrated that an appetitive or an aversive behavioral state has an opposite effect on the plastic properties of VTA DA excitatory synapses in a projection specific manner. Notably, although it is still controversial, the scientific community has accepted that specific SNpc or VTA DA sub-populations, each with specific projection target areas and differential afferent inputs, might have different integrative electrophysiological properties and behavioral functions [42].

Anatomical Organization and Molecular Diversity of the Midbrain DA Neurons

Morphology of Midbrain DA Neurons

Within the VTA and the SNpc, DA neurons expressing tyrosine hydroxylase (TH, i.e. the rate-limiting enzyme in DA synthesis) are interspersed with GABAergic neurons [43, 44]. Importantly, a population of glutamatergic neurons in the VTA has also been identified [45, 46]. DA neurons are medium to large size cells with a body diameter ranging from 12 to 25 μm [47, 48]. They display morphological variations in their shape (i.e. ovoid, medium sized or fusiform) and in the orientation of their dendritic arborization [48], which suggest a degree of diversity at least among SNpc DA neurons. DA neurons emit sparse and relatively unbranched dendritic arborization. Most of their dendrites are smooth and/or occasionally and sparsely invested

with “spine-like appendages” [48]. Anatomical evidence suggests that SNpc DA neurons projecting to the striatum could be under modulation of the VTA, since they extend their dendritic arborization in this neighboring area [48]. These findings support the notion that SNpc and VTA are part of a single continuous cell group, rather than two separate nuclei [49, 50].

Molecular Diversity of Midbrain DA Neurons

The VTA and the SNpc are two highly heterogeneous brain structures in terms of their neuronal phenotype. In the VTA and SNpc, the DA neurons represent 65 and 70% of the total number of neurons, respectively [43]. Moreover, *in situ* hybridization and RT-qPCR coupled to laser-capture approaches revealed distinct populations of DA neurons spread across the VTA and SNpc [30, 43, 46].

Key markers of DA neurons include mRNA for TH, DA transporter (DAT), vesicular monoamine transporter 2 (VMAT2), G-protein-coupled inwardly rectifying potassium channel subunit 2 (GIRK2), DA D2 receptor (D2) and vesicular glutamatergic transporter type 2 (vGluT2). The abundance of these biochemical markers co-vary according to the localization of the DA neuron within the VTA or the SNpc, and to their projection targets.

The identification of the molecular diversity of DA neurons is primarily important for the unraveling of DA system function. In addition, the identifying of this variety is key for a proper interpretation of findings obtained with optogenetics, which accesses and controls network activity at a single cell level by using cell-type *specific promoter* elements [42]. For instance, 80% of the TH immunolabeled neurons in the medial part of the VTA were negative for the transcript encoding DAT [46], thus suggesting that optogenetic approaches targeting the DAT promoter only manipulate a subset of DA neurons. Additionally, 82% of the TH immunolabeled neurons in the anterior part of the VTA were negative for the transcript encoding DAT, and did not release DA in their terminal region (i.e. lateral habenula [51]). Consequently, it appears that neither targeting the TH promoter with optogenetics always and necessarily results in modifications in DA release in the target region. Remarkably, current evidence suggests that molecular diversity is more evident among DA neurons in the VTA rather than in SNpc [42]. Hence, functional diversity has been often reported between posterior versus anterior, and lateral versus medial, DA neurons *in vivo* [29, 30, 41, 52].

Physiology of Midbrain DA Neurons

A better understanding of the functions of midbrain DA neurons derives from the pioneering electrophysiological studies carried out by performing single unit intra- and extra- cellular recordings *in vivo* [53–58]. At first, DA neurons were identified and characterized within the SNpc [53]. In rodent and awake nonhuman primate, VTA and SNpc DA neurons recorded *in vivo* display a characteristic electrophysi-

ological signature that allows them to be distinguished from non-DA neurons in surrounding regions of the VTA or in the pars reticulata of the substantia nigra (SNpr) [55, 56, 59–61]. Subsequently, these electrophysiological features have been re-examined and reviewed [62]. The current criteria for their identification include: (i) an action potential with biphasic or triphasic waveform, with a duration longer than 2.2 ms; (ii) a slow spontaneous firing rate (2–9 Hz); (iii) a regular and/or burst spontaneous firing pattern, this latter being characterized by a spike-amplitude decrement; (iv) the inhibition of spontaneous activity following administration of DA D2 receptor agonists, and subsequent reversal by DA D2 receptors antagonists.

Midbrain DA neurons fire bursts of action potentials in response to sensory stimuli leading to transient increases in extracellular DA levels in target areas [63]. *In vivo* DA neurons fire bursts of action potentials upon depolarization of their membrane and display pauses after GABA conductance activation [64]. Bursts are supposed to mediate reward information coding, whereas pauses signal the absence of an expected reward. Accumulating evidence shows that an increased glutamatergic or cholinergic drive produces the characteristic bursting pattern observed *in vivo* [65–67], and that NMDARs in DA neurons modulate burst firing and DA release in postsynaptic brain regions [68]. Furthermore, bursting activity of DA neurons is sufficient to mediate behavioral conditioning in freely-behaving rats [69]. Conversely, phasic changes in tonic activity of GABAergic afferents are a potential extrinsic mechanism able to trigger bursts and pauses in DA neurons [64]. Consequently, DA neurons are regulated by an integrated network of inputs [70], and their firing pattern is sculpted through the balance of activation of GABAergic inputs and NMDA receptor-mediated excitatory inputs.

Connectivity of SNpc and VTA DA Systems

Midbrain DA ascending pathways are organized in three major tracts (Fig. 17.1). The mesolimbic and mesocortical pathways project from the VTA to the NAcc, limbic areas, i.e. amygdala, septal area, bed nucleus of the stria terminalis (BNST), and the PFC, and are mainly implicated in associative learning, reward signaling and goal directed behavior [26, 28, 71–73]. The nigrostriatal pathway projects from the SNpc to the dorsal striatum, and is primarily involved in the regulation of motor activity, exploration and action selection [42]. Most of the knowledge regarding the mapping of inputs to VTA and SNpc DA neurons derives from classical tract-tracing studies [74] and viral-based tracing approaches [70] (Fig. 17.2).

Only a small proportion of cortical excitatory inputs innervate VTA and SNpc DA neurons [70]. Notably, VTA DA neurons receive fewer cortical inputs when compared with SNpc DA neurons. Cortical inputs to SNpc DA neurons are mostly coming from the primary and secondary motor cortices, whereas the only major cortical projection to the VTA originates from the PFC (mainly prefrontal and infralimbic cortices) [70, 75]. The BNST sends most of its projections to VTA DA neurons [76–78], and less to SNpc DA neurons [70]. From the midbrain and hind-brain, VTA and SNpc DA neurons receive the largest input from the superior col-

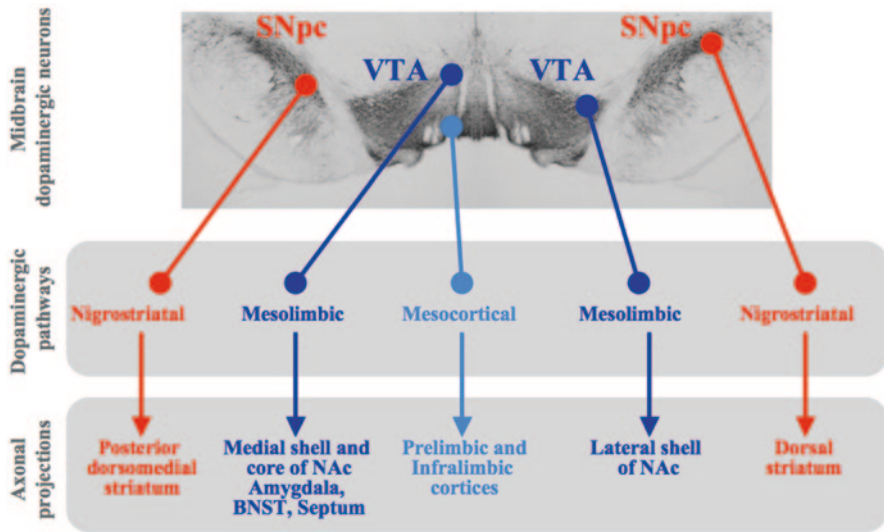


Fig. 17.1 Schematic organization of the three major midbrain ascending pathways. The mesolimbic and mesocortical pathways project from the ventral tegmental area (*VTA*) to the nucleus accumbens (*NAc*), limbic areas such as amygdala, septum, bed nucleus of the stria terminalis (*BNST*) and the prefrontal and infralimbic cortices. The nigrostriatal pathway projects from the pars compacta of the substantia nigra (*SNpc*) to the dorsal striatum

liculus, the periaqueductal gray (PAG) and the dorsal raphe (DR). The pedunculo- tegmental nucleus (PPTg) preferentially projects to the SNpc DA neurons, whereas laterodorsal tegmental nucleus (LDTg) preferentially projects to VTA DA neurons. The dorsal striatum (DS), the external globus pallidus (GPe) and the SNpr are major inhibitory GABAergic inputs controlling SNpc DA cell activity. Within the mid- brain, the tail of the ventral tegmental area (tVTA) [79], also named the rostromedial tegmental nucleus (RMTg) [80], appears as a GABAergic inhibitory structure heavily projecting to the VTA and SNpc. Hence, the tVTA/RMTg regulates the activity of midbrain DA systems [81, 82]. In summary, although the input-output connectome of midbrain DA neurons has been partially elucidated at the structural level, the connectivity at the level of individual DA neuron requires to be further explored [83].

Integrative Properties of SNpc and VTA DA Neurons

Change in Excitability: Integration and Balance Between Inhibitory and Excitatory Inputs

Similar to other neuronal types, DA cell dendrites receive information from tens of thousands of synaptic inputs. This information are coordinated and stored via highly complex processes of dendritic integration of both inhibitory and excitatory

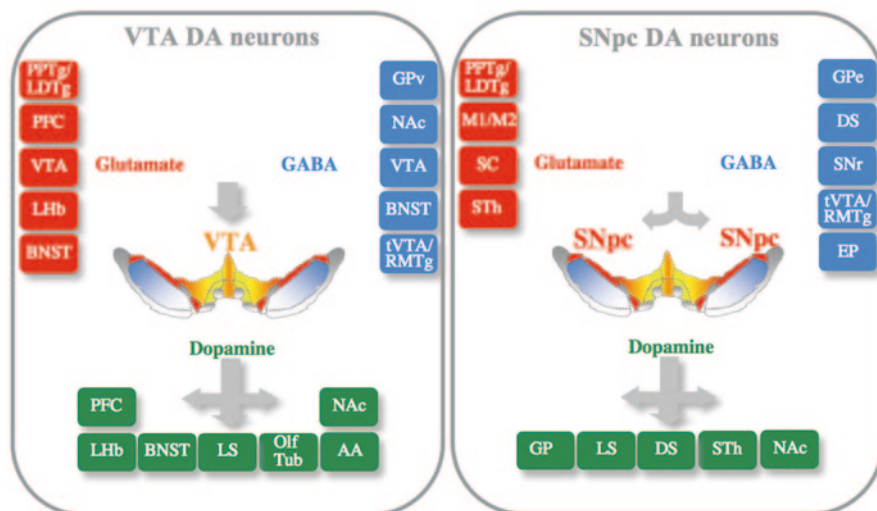


Fig. 17.2 Schematic organization of the principal afferents and efferents to the VTA (*left*) and SNpc (*right*) dopamine neurons. For clarity, only some of the projection are shown. *Red* indicate excitatory glutamatergic structure, *blue*, inhibitory GABAergic structures and *green*, dopaminergic target areas. Glutamatergic and GABAergic control the excitability of VTA and SNpc dopaminergic neurons. The pedunculotegmental nucleus (*PPTg*) preferentially projects to SNpc dopaminergic neurons, whereas laterodorsal tegmental nucleus (*LDTg*) preferentially projects to VTA dopaminergic neurons. The VTA dopaminergic neurons receive fewer cortical inputs than SNpc dopaminergic neurons. *SC* superior colliculus, *PAG* the periaqueductal gray, *DR* dorsal raphe, *DS* The dorsal striatum, *GPe* external globus pallidus tVTA/RMTg tail of the ventral tegmental area/rostromedial tegmental nucleus. There are only a few overlaps between dopaminergic efferents from the VTA and the SNpc. *PFC* prefrontal cortex, *LHb* lateral habenula, *BNST* bed nucleus of the stria terminalis, *LS* lateral septum, *OlfTub* olfactory tubercle, *AA* amygdala, *NAc* nucleus accumbens, *STh* subthalamic nucleus, *GP* globus pallidus, *DS* dorsal striatum

synaptic inputs. This integrative computation can influence sub-threshold membrane potentials, and play a role in the switch between tonic and phasic DA signal. Endocannabinoids, by serving as retrograde messengers and key modulators of synaptic functions, participate in this switch [2]. Indeed, they can finely tune firing activity and pattern of VTA DA neurons [84, 85] and, consequently, their phasic DA release in the NAc [86]. Hence, the emerging picture is that the endocannabinoid system acts as a local device for DA neurons to switch their firing pattern and activity in response to stimuli not only in the VTA [84, 85] but also in the SNpc [23, 24]. Given that physiological significance of endocannabinoid signaling at synapses onto DA neurons is reflected in the activity of these cells in response to input stimulation, these lipid molecules ultimately finely tune phasic versus tonic, and *vice versa*, DA release in the terminal regions [86, 87]. These considerations highlight how powerfully the endocannabinoid system might regulate not only DA volume transmission, but also DA modulation of cortical and subcortical information processing. In addition, they help explaining the correlation between an unbalanced

endocannabinoid signal and altered DA-dependent processes underpinning diverse pathological conditions of both nigrostriatal and mesocorticolimbic systems [4].

Role of Endocannabinoids on GABA Afferents

As already mentioned, a tight regulation of DA levels in terminal regions is crucial as DA regulates key features of motivated behaviors to provide the adaptability of behavioral outputs required for species survival, such as for instance approach towards and withdrawal from rewarding and aversive stimuli, respectively [71, 88, 89].

DA neuronal activity results from the finest regulation of intrinsic and extrinsic mechanisms. Both VTA and SNpc DA neurons are subject to major background GABA inputs, whose activation results in either inhibition of their spontaneous activity (in terms of both firing activity and number of active cells) and/or in triggering bursts and pauses in DA cells [64, 90]. As a result, the dissection of which synapse is equipped with molecular architecture of a given endocannabinoid is extremely relevant from a functional point of view.

From an anatomical point of view, CB₁ receptors are located on GABAergic synapses onto VTA and SNpc DA cells [6, 8]. In the VTA, 2-AG biosynthetic enzyme diacylglycerol (DAG) lipase is found in DA cells at the level of the plasma membrane, and the main degrading enzyme monoacylglycerol (MAG) lipase is localized at a presynaptic level [6]. In the SNpc, instead, the molecular determinants for 2-AG have not been identified yet. In addition, anatomical evidence indicates the presence of the enzyme N-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD) within the midbrain [91], thus supporting the notion that *N*-acylethanolamines such as anandamide and endogenous ligands to peroxisome proliferator-activated receptor- α (PPAR α) (i.e. oleoylethanolamide, palmitoylethanolamide) are abundant under basal conditions in midbrain slices [1, 10].

Notably, the endocannabinoid/vanilloid *N*-arachidonoyl-dopamine (NADA) can be also detected within the SNpc, but only upon K⁺-induced depolarization or activation of postsynaptic metabotropic glutamate receptor type-1 (mGluR1) on SNpc DA cells [8, 24]. Hence, in the SNpc, electrophysiological evidence points to a role of tonic NADA released by DA cells upon mGluR1 activation resulting from glutamate spillover from nearby synapses likely arising from subthalamic nucleus [24]. Thus, concomitant activation of excitatory inputs might be associated to endocannabinoid-mediated inhibition of GABAergic afferents, thereby enhancing DA cell responsiveness to excitatory stimuli and resulting in burst firing [24]. In this scenario (i.e. SNpc), the endocannabinoid produced by DA cells *on demand* (i.e. cell membrane depolarization, activation of postsynaptic muscarinic receptors) [23, 24] is most likely 2-AG, which mediates depolarization-induced suppression of inhibition (DSI), a short-term form of synaptic plasticity. Particularly, 2-AG would activate CB₁ receptors on striatonigral terminals, which in turn decrease GABA release and lead to intrinsic inhibition of DA cells [23]. Notably, Yanovsky et al. [23] questioned the canonical mode of action, and described for the first time a tonic

endocannabinoid tone at these synapses. Hence, they suggested that endocannabinoids and DA are co-released within the SNpc to modulate striatonigral GABAergic fibers through the activation of CB₁ and D1 receptors, respectively. Remarkably, both DA- and endocannabinoid- induced changes in inhibition occur independently from each other. Indeed, activation of D1 receptors by somato-dendritically released DA would emphasize extrinsic inhibition to the expenses of a decreased intrinsic inhibition due to the counteraction of DSI. On the other hand, CB₁ receptor activation would promote intrinsic inhibition [23]. Given that both D1 and CB₁ receptors are located on striatonigral fibers, whereas D1 receptors are only on pallidonigral fibers but not on intrinsic fibers, co-release of DA and endocannabinoids might most likely occur upon different scenarios, such as discrete types of discharge rates and patterns of DA neurons. This would ultimately allow for a differential tuning of inhibition of DA cells within SNpc, and would be consistent with the spatio-temporal definition of endocannabinoid actions at synapses [23] (Fig. 17.3).

In the VTA, electrophysiological evidence converges to a role of 2-AG in modulating GABA inputs, whereas there is no indication supporting a role for either anandamide or NADA in regulating plasticity at these synapses [85, 92, 93]. Indeed, either intracellular loading of DAG lipase inhibitors or G-protein inhibitors into DA neurons proved to block endocannabinoid- mediated actions on discrete GABA receptors [85, 92, 93]. These observations are supported by the localization of 2-AG-synthesizing enzyme DAG lipase in the DA cell [6], which would release 2-AG following group I mGluR activation [92]. Noteworthy, release of 2-AG by VTA DA cells would occur under conditions similar to those of SNpc DA cells. Particularly, it has been shown that under conditions favoring bursting 2-AG decreases either GABA_A- and/or GABA_B- mediated responses of VTA DA cells [85, 92]. In fact, activation of group I mGluRs or increased intracellular Ca²⁺ concentrations, and block of small conductance calcium-activated potassium channels (SK channels) [85, 94] can lead to 2-AG production and its dependent effects [92]. Thus, DA neuron responsiveness to excitatory input would increase, and in the presence of a diminished inhibition, the balance of activity might most likely shift toward excitation and bursting [95, 96] (Fig. 17.4). Notably, sex differences in 2-AG effects have been revealed in the rat VTA [93]. In particular, electrophysiological evidence suggests that female rats displayed a tonic 2-AG signaling acting upon CB₁ receptors onto discrete GABA inputs onto VTA DA cells. Noteworthy, no sex dichotomy is found in CB₁ receptor expression and function in the VTA, whereas activation effects of sex hormones regulate the density of these receptors [97]. In particular, CB₁ receptor density decreases in both the PFC and amygdala of female rats. Noteworthy, both of these regions are critically involved in decision-making and learning processes underlying goal-directed behaviours [98, 99], in which important gender differences have been described [100]. Thus, such an enhanced 2-AG tone onto inhibitory inputs onto VTA DA cells might contribute not only to disinhibition of DA neurons, but also to sex-dependent differences in their function under normal and pathological conditions even before gonadal function fully matures [101–103]. This is in agreement with the increasing number of studies [101, 104–106] highlighting that sex/gender differences of brain structure and network function are not simply restricted to structures primarily involved in sexual behavior (see also Chap. 12).

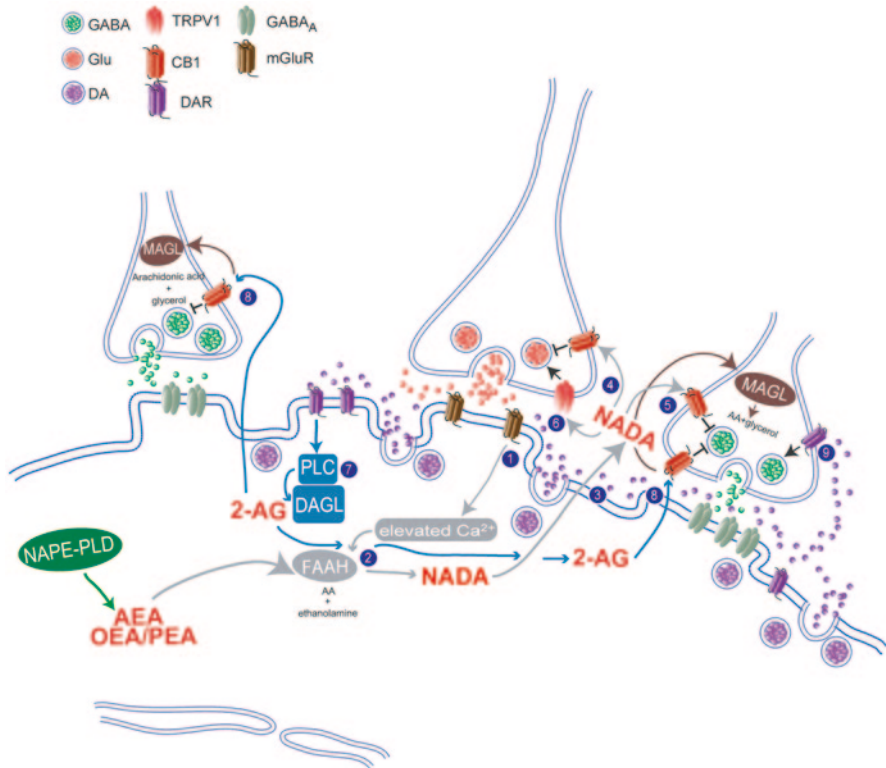


Fig. 17.3 Schematic organization of endocannabinoid system onto dopamine neurons of the Substantia Nigra pars compacta. Proposed mechanism by which endocannabinoids, namely 2-arachidonoylglycerol (*2-AG*) and N-arachidonoyldopamine (*NADA*), regulate synaptic transmission onto dopamine (*DA*) neurons of the pars compact of Substantia Nigra (*SNpc*) are illustrated. Glutamate spillover activates postsynaptic type 1 metabotropic glutamate receptors (*mGluR*) (1) [24] with consequent increase in intracellular Ca^{2+} (2) [24], thus leading to generation of *NADA*. Arachidonic acid for *NADA* synthesis is provided by the *FAAH*, which appears to be directly involved in *NADA* synthesis [186]. Subsequently, *NADA* binds to presynaptic CB_1 receptors expressed on glutamate (4) [8] and GABA terminals (5) [8, 24] to reduce neurotransmitter release. *NADA* can also bind *TRPV1* receptors located on glutamatergic terminals (6) [8] and finely tune *SNpc* *DA* neuron activity. Activation of postsynaptic dopamine D_2 (*DAR*) cooperates to enhance *2-AG* synthesis. *2-AG* is produced *on demand* via a Ca^{2+} -independent mechanism involving activation of phospholipase C (*PLC*) (7) [24], which in turn cleaves phosphatidylinositol 4,5-bisphosphate (*PIP2*) into inositol trisphosphate (*IP3*) and *DAG*. *DAG* is then hydrolyzed by diacylglycerol lipase (*DAGL*). *2-AG* moves retrogradely across the synaptic cleft to activate CB_1 receptors located on GABAergic terminals (8) [23]. A subset of presynaptic GABAergic terminals (i.e. striatonigral) co-express DAD1 receptors (*DAR*), whose activation by somatodendritically released *DA* counteracts *2-AG* effects (9) [23]. The strengthening of extrinsic inhibition in the network reduces local (i.e. intranigral) inhibition. The two discrete retrograde signaling mechanisms (i.e. *2-AG* and *DA*) operate independently, and their functional need remains elusive. However, the co-expression of *DAR* and CB_1 receptors on striatonigral fibers, while *DAR* are exclusively present on pallidonigral fibers and absent in intranigral fibers, allows the two retrograde signals for changes the influence of intrinsic versus extrinsic inhibition [23]. Whether these terminals are equipped with the monoacylglycerol (*MAG*) lipase (*MAGL*) has to be established yet.

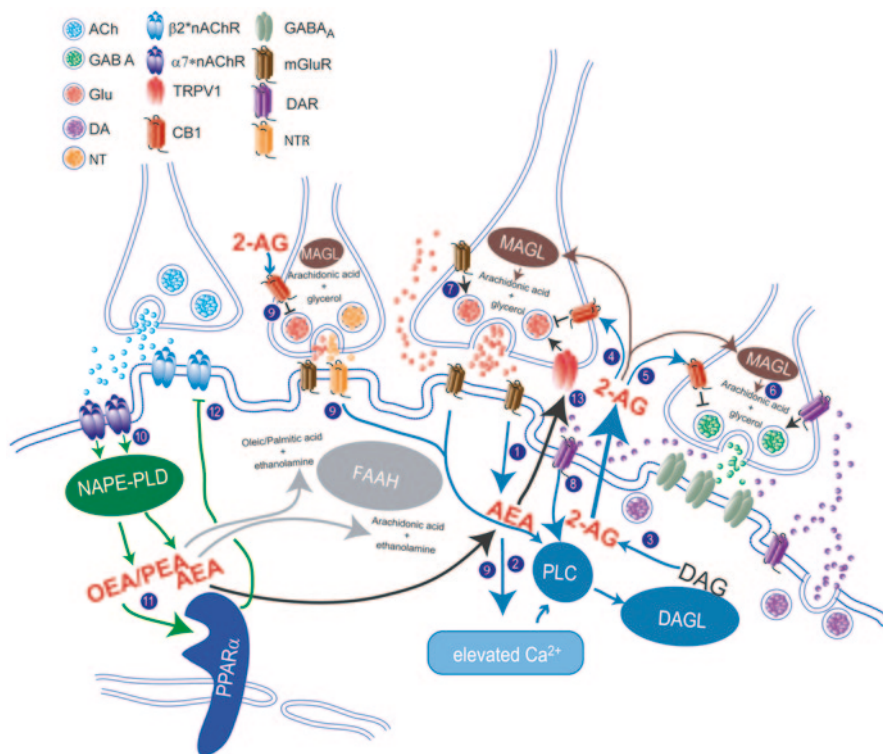


Fig. 17.4 Schematic organization of endocannabinoid system onto dopamine neurons of the ventral tegmental area. Proposed mechanisms by which endocannabinoids (i.e. 2-arachidonoylglycerol and anandamide) and N-acyl ethanolamines (OEA and PEA) regulate synaptic transmission onto ventral tegmental area (VTA) dopamine (DA) neurons are illustrated. Anandamide (AEA) and 2-arachidonoylglycerol (2-AG) are produced *on demand* by two Ca^{2+} -dependent enzymes, which are N-acylphosphatidylethanolamine hydrolyzing phospholipase D (NAPE-PLD) and diacylglycerol lipase (DAGL), respectively. Activation of metabotropic glutamate receptors (*mGluR*) (1) [84] increases intracellular Ca^{2+} (2) [84, 85] that activates phospholipase C (PLC), which in turn cleaves phosphatidylinositol 4,5-bisphosphate (PIP₂) into inositol trisphosphate (IP₃) and DAG [84, 154]. DAG is then hydrolyzed by DAGL to generate 2-AG (3) [84]. 2-AG binds to presynaptic CB₁ receptors expressed on glutamate (4) [84, 154] and GABA terminals (5) [85, 93, 187] to depress neurotransmitter release [84, 154]. 2-AG is mainly degraded by the enzyme MAG lipase (MAGL) expressed in both glutamatergic and GABAergic terminals (6) [6, 93]. Presynaptic activation of mGluRs on glutamatergic terminals concurs with 2-AG to dampen further excitability of DA cells by decreasing probability of glutamate release (7) [85]. Activation of postsynaptic dopamine D₂ (DAR) cooperates to enhance 2-AG synthesis and release (8) [154, 187]. Activation of post-synaptic neurotensin receptors (NTR) leads to G-protein dependent activation of PLC, and consequent generation of PIP₂, IP₃ and DAG (9) [115]. Subsequent hydrolysis of DAG by DAGL generates 2-AG, which then moves retrogradely across the synaptic cleft to activate CB₁ receptors located on a subset of glutamatergic terminals co-releasing NT, thus resulting in inhibition of glutamate and NT release [115]. Whether these terminals are equipped with MAGL has to be established yet. AEA is synthesized, along with OEA and PEA, by NAPE-PLD (10). AEA activates TRPV1 receptors located on presynaptic glutamatergic terminals (13) [154], whose activation leads to increased spontaneous activity of DA cells [87, 113]. OEA and PEA are instead endogenous ligands of type α Peroxisome proliferator-activated receptors (PPAR α), whose activation

Although immunocytochemical investigation of CB₁ receptors failed to precisely identify the origin of GABAergic afferents [6], electrophysiological evidence suggests CB₁ receptors to be localized onto those inputs arising from ventral pallidum [85, 93, 107], RMTg nucleus [93, 108] and local GABA neurons [109]. Given that these discrete inputs play important roles in controlling the number of spontaneously active DA neurons [90] and their own discharge rate [108, 110, 111], it is paramount to examine whether these synapses are differently equipped/enriched with discrete players of 2-AG signalling machinery. Hence, since GABA removal induces disinhibition bursts [64], the endocannabinoids NADA and 2-AG might be likely involved in transiently silencing inhibitory synapses onto midbrain DA cells, thereby contributing to their phasic excitation in the framework of multiple signalling modalities.

Role of Endocannabinoids on Glutamatergic Afferents

Endocannabinoid modulation of excitatory synapses impinging upon midbrain DA neurons has been extensively investigated within the past decade. CB₁ receptors have been found to be located on glutamatergic/asymmetric synapses in the SNpc and VTA [6, 8], where they serve as targets for endocannabinoids. Particularly, CB₁ receptors have been identified more abundantly on vesicular glutamate transporter 1 (VGLUT1)-positive terminals, predicted to be of cortical origin, rather than on VGLUT2-expressing terminals, expected to be of subcortical origin [74], in close proximity to VTA DA neuron dendrites [7].

To date, SNpc and VTA DA cells appear to synthesize and release diverse endocannabinoids acting on different targets, in agreement with their spatial definition. For instance, in the SNpc, 2-AG has not been reported to modulate excitatory inputs onto DA neurons. In contrast, anandamide was first shown to be released upon K⁺-induced depolarization and to enhance glutamatergic inputs via activation of ionotropic transient receptor potential vanilloid type 1 (TRPV1) receptors [112]. Subsequently, NADA was also shown to modulate DA cell excitability through activation of either TRPV1 or CB₁ receptor [8]. Importantly, under basal conditions NADA concentrations are barely detectable within the SNpc [8, 24], thus supporting the hypothesis that it could be released only upon particular functional states of DA cells. Since NADA synthesis depends upon DA synthesis itself, a plausible scenario might be that under basal conditions low levels of NADA are released to preferentially activate TRPV1 receptors and enhance glutamate release. Instead,

(11) [113, 124] leads to phosphorylation and, thereby, negative modulation of somatodendritic nicotinic acetylcholine receptors containing the $\beta 2$ subunit ($\beta 2^*nAChRs$) (12) [10, 113, 124]. Synthesis of OEA and PEA depends upon activation of somatodendritic nAChRs containing the $\alpha 7$ subunit ($\alpha 7nAChRs$) as well as increases in intracellular Ca²⁺ (10) [10]. The increased levels of OEA and PEA acting via PPAR α serve to prevent aberrant hypercholinergic-driven excitation of DA neurons (12) [10]. AEA, OEA and PEA are mainly degraded by the fatty acid amide hydrolase (FAAH) located on the postsynaptic compartment [6]

upon either prolonged or excessive depolarization, NADA binds to CB₁ receptors to dampen glutamatergic transmission [8] (Fig. 17.3). Whether a similar scenario applies to the neighboring cells of the VTA is still not determined, although anandamide excites these cells via TRPV1 [113], whose activation results in increased DA levels in the NAc [87].

In the VTA, CB₁ receptors are localized on asymmetric synapses at the opposite site of the main synthesizing enzyme for 2-AG, that is the DAG lipase [6], within the DA cells. Several lines of evidence indicate 2-AG as the key endocannabinoid released *on demand* by VTA DA neurons, which mediates both short- and long-term forms of synaptic plasticity. Particularly, 2-AG mediates depolarization-induced suppression of excitation (DSE) [9], a form of short-term plasticity that most likely serves to limit pathological excitation of DA neurons, such as that observed under ischemic-reperfusion injury [9]. Additionally, 2-AG is released by DA neurons during behaviourally relevant patterns of synaptic activity, such as a brief burst of excitatory synaptic activity [84]. Under these conditions, mGluR1 activation and enhanced intracellular Ca²⁺ levels contribute to its synthesis and release, ultimately leading to transient and selective silencing of excitatory inputs onto the neuron itself. Thus, 2-AG ensures a fine modulation of both spike and burst probability of VTA DA cells [84] (Fig. 17.4).

2-AG also plays a role in diverse forms of long-term synaptic plasticity. Particularly, it mediates long-term depression (LTD) [114, 115], and negatively gates long-term potentiation (LTP) at these synapses [7]. Indeed, low frequency stimulation (LFS)-induced LTD requires 2-AG, since pharmacological inhibition of either phospholipase C (PLC) or DAG lipase, both critical for 2-AG biosynthesis, abolished LFS-LTD, whose induction also necessitates an increased postsynaptic intracellular Ca²⁺ through L-type Ca²⁺ channels [114]. Another form of 2-AG-mediated LTD is expressed by VTA DA cells, and this is the insulin-dependent LTD [116]. Indeed, acute activation of insulin receptors is able to induce a Ca²⁺-independent release of 2-AG, which binds to and activates presynaptic CB₁ receptors located selectively on glutamatergic terminals, and induces LTD [116]. Notably, 2-AG also mediates LTD in response to activation of neurotensin (NT) receptors [115]. Hence, it appears that under conditions resulting in a significant release of NT in the VTA, NT co-released with glutamate from VGLUT-positive axon terminals could negatively regulate excitatory inputs onto DA neurons and, therefore, LTP induction. Notably, NT-induced long lasting depression of glutamate release via 2-AG requires Gq-protein-mediated activation of PLC and subsequent DAG-lipase activity, but not raises in Ca²⁺ levels. In turn, 2-AG via CB₁ receptor activation reduces glutamate release by inhibiting voltage-dependent Ca²⁺ channels (VDCC) [115] (Fig. 17.4). This finding is particularly relevant from a pathophysiological perspective. Indeed, the observations that NT CSF levels are dramatically reduced in drug-free schizophrenic patients, and that an altered NT neurotransmission within the VTA can be associated with mesolimbic DA hyperactivity characteristic of schizophrenia [117], highlight the relevance of such a tight and long lasting regulation of DA cell excitability by NT via CB₁ receptors. Remarkably, 2-AG, released by DA neurons and through activation of CB₁ receptors on VGLUT1-positive terminals, also negatively regulates

spike time-dependent LTP induction [7]. Thus, it appears that under circumstances of strengthened excitatory plasticity, DA cells would release 2-AG, which mediates LTD and impairs LTP at the same synapses to protect DA cells from aberrant excitation, while simultaneously silencing inhibitory afferents [92]. Altogether, these findings might help elucidating the paradox, and explain the controversy, that exposure to *Cannabis* might be either a self-medication for psychotic patients and psychomimetic [118, 119]. Hence, one has always to take into account that differences exist between the effects of CB₁ receptor activation by endogenous ligands and CB₁ receptor agonists (e.g. *Cannabis*, spice drugs), and, finally yet importantly, that endocannabinoid system states may differ among and within individuals.

Role of Endocannabinoids on Cholinergic Afferents

Midbrain DA cell firing and pattern are also powerfully controlled by extrinsic cholinergic inputs arising from the LDTg and the PPTg nuclei [120] through activation of nicotinic receptors (nAChRs) [121, 122]. Midbrain DA cells express two major forms of nAChRs, high-affinity $\beta 2^*$ -nAChRs and low-affinity $\alpha 7$ -nAChRs [123], where $\beta 2^*$ -nAChRs enable the transition from tonic to phasic activity [122]. Remarkably, $\beta 2^*$ -nAChRs can be negatively modulated by endogenous ligands of PPAR α , such as *N*-acylethanolamines and fatty acids [1, 10, 124]. Notably, the *N*-acylethanolamines oleoyl-ethanolamide (OEA) and palmitoyl-ethanolamide (PEA) are regarded as belonging to the “extended family” of endocannabinoids [125]. In fact, both the enzyme FAAH and NAPE-PLD, key in degradation and synthesis of the endocannabinoid anandamide, tightly regulate levels of other *N*-acylethanolamines along with anandamide [126, 127]. Thus, endogenous PPAR α ligands, such as the anorectic OEA [128] and the anti-inflammatory PEA [129], by sharing with anandamide both the anabolic and degradative pathway [130] can produce an indirect activation of other receptors and the so-called ‘entourage effect’ [131–134].

N-acylethanolamines, by activating PPAR α , decrease spontaneous activity of VTA DA cells and the number of spontaneously active DA neurons through a rapid non-genomic mechanism [10, 124]. These effects, rapid in onset and blocked by the tyrosine kinase inhibitor genistein [113], are indicative of phosphorylation of $\beta 2^*$ -nAChRs as the underlying mechanism of PPAR α -mediated actions [10, 124]. *N*-acylethanolamines are found in all mammalian tissues [135] and are abundantly present within the midbrain [1, 10], where they enable VTA DA cells to switch between tonic/phasic modes of activity that are tightly regulated by $\beta 2^*$ -nAChRs [10, 122]. Additionally, their synthesis and/or release occurs *on demand* upon $\alpha 7$ -nAChRs activation [10]. Hence, in VTA DA neurons, acetylcholine and *N*-acylethanolamines appear to control each other in a negative feedback mechanism, where high acetylcholine enhance OEA and PEA levels to negatively modulate $\beta 2^*$ -nAChRs downstream to PPAR α activation in order to prevent aberrant DA cell excitation [10] (Fig. 17.4). Thus, by modulating VTA DA cell excitability, PPAR α may have consequences for a number of behavioral responses known to be sensitive to the function of DA circuits. Since physiological activation of these neurons occurs across three

dimensions that affect firing rate, firing pattern and the proportion of spontaneously active neurons, and that PPAR α activation has been shown to affect at least two of these [124], it is very important to further examine the role of these nuclear receptors within the midbrain. Particularly, given the prominent categorical difference between SNpc and VTA DA neurons with respect to energy metabolism [136], one could speculate that some might be less susceptible to metabolic distress. This is particularly important because mitochondrial dysfunction appears to be critical to the pathogenesis of sporadic Parkinson's disease, which is due to degeneration of DA neurons within the SNpc [137] whereas those within the VTA are spared. Therefore, whether or not DA cells within the SNpc are under PPAR α regulation similar to the VTA is a critical issue.

Physiological Events Triggering the Release of Endocannabinoids in Midbrain DA Regions

The aforementioned molecular machinery for 2-AG negative feedback pathway is remarkably conserved at both glutamatergic and GABAergic synapses in the VTA [6], and most likely in the SNpc. However, a prominent role for NADA has been described exclusively within the SNpc [8, 24]. Thus, it appears that endocannabinoids, by regulating DA cell activity either in an homo- or hetero- synaptic fashion, may not only contribute to the rewarding/teaching signal encoded by these neurons, but may also regulate the start/stop of sequence learning [138]. Additionally, endocannabinoids may participate in discrete mechanisms aimed at DA cell homeostatic regulation. As a result, given the role of the endocannabinoid system in modulating DA neuronal function, they might take part in motor skill learning and in the action-habit transformation. Hence, several and diametrically opposite events in life are able to trigger endocannabinoid synthesis and release from midbrain DA cells, such as for instance the pursuit of natural rewards, physical exercise, stress and noxious stimuli.

Endocannabinoids have long been involved in appetitive-motivational aspects of reward-directed behaviors [139–141]. The first demonstration that 2-AG is the endocannabinoid enhancing neural mechanisms of cue-motivated reward seeking, thereby supporting its key role for multiple forms of synaptic plasticity within the VTA [2], has been elegantly provided by Cheer's group [142]. In particular, the Authors showed the existence of a single neural signaling mechanism through which CB₁ receptor antagonists can effectively reduce the influence that environmental cues exert over motivated behavior. Indeed, it is well established that the motivational state of the individual regulates those appetitive behaviors involving the pursuit of reward [143, 144]. Thus, Oleson and colleagues demonstrated that the disruption of 2-AG signaling in the VTA simultaneously decreases cue-evoked reward seeking as well as DA levels in the NAc shell [142]. Conversely, pharmacological enhancement of 2-AG signaling (i.e. by inhibition of its main degrading enzyme) within the VTA produces the opposite behavioral output. Therefore, the Authors

indicate 2-AG in the VTA as the main endocannabinoid involved in mediating cue-motivated reward directed behaviors [142]. Notably, several lines of evidence postulate that cue-encoding VTA DA cells form discrete neural assemblies with GABAergic synapses, which will consequently allow for a fine-tuning of DA neural activity itself during reward seeking. Accordingly, activation of CB₁ receptors located on discrete GABAergic terminals have been shown to decrease GABA release onto VTA DA neurons [85, 93, 107–109], thereby resulting in their disinhibition.

Similarly, it has been proven that physical exercise enhances the endocannabinoid system in both humans [145] and rodents [32, 146–148], and that its involvement may take part in beneficial effects of exercise. Notably, the reinforcing properties of wheel running are ascribed to activation of the endocannabinoid system on wheel-based seeking which, similarly to the pursuit of reward, is a form of appetitive behavior [148]. Remarkably, activation of CB₁ receptors on GABAergic terminals onto VTA DA cells exerts a tonic stimulatory influence on voluntary running performance [147]. Particularly, an endocannabinoid-dependent stimulation of CB₁ receptors located on VTA GABAergic terminals can define running performance as endocannabinoids regulate DA-dependent reward-directed processes. In turn, both acute and repeated voluntary exercise considerably affect VTA DA cell activity [147]. Particularly, following acute and repeated voluntary wheel running GABA-CB₁^{-/-} mice display a marked decline in the number of spontaneously active DA neurons, which also show a reduction in both firing rate and bursting activity. Although the nature of the endocannabinoid exerting this tonic control on inhibitory transmission via CB₁ receptor activation has to be elucidated yet, this elegant study highlights and remarks the role of this endogenous system in the regulation of VTA DA neuron activity after both acute and repeated voluntary running. In this scenario, shortly after running the absence of CB₁ receptors at inhibitory synapses shifts excitation towards inhibition of DA neuronal activity, and their behavioral output [147]. Consistent with these findings are the observations that CB₁ deficient mice exhibit dramatic motor deficits [149], and lack endocannabinoid-dependent LTD at the indirect pathway within the basal ganglia [150].

Excitatory synaptic transmission onto VTA DA cells is also potentiated by feeding-related peptides [151, 152], and this might underlie the motivation to obtain food [151, 153]. On the other hand, insulin in the VTA might reduce the salience of cues and/or contexts associated with food by reducing the strength of excitatory synapses onto DA neurons via 2-AG [116]. In fact, electrophysiological observations suggest that when plasmatic levels of insulin are increased, such as immediately following a caloric meal consumption (i.e. sweet high fat meal), 2-AG levels increase within the VTA, where its effects are unmasked by pharmacological blockade of CB₁ receptors [116]. Thus, it appears that insulin receptor activation triggers 2-AG synthesis and release from VTA DA neurons to activate CB₁ receptors on a subset of excitatory terminals. This results in a decreased glutamate release from presynaptic terminals without affecting GABA transmission [116], in according to the spatial definition of endocannabinoid actions. Notably, these findings do not reflect the effort exerted by the animal to obtain a palatable food, but they show that enhanced circulating levels of insulin via CB₁ receptor activation may reduce

simple appetitive behaviors displayed routinely before food consumption as well as the salience of food-related cues [116].

As often mentioned in this chapter, midbrain DA neurons are potential indicators of salient, pleasant, or noxious stimuli, and they are tightly regulated by both excitatory and inhibitory inputs equipped with both CB₁ and/or TRPV₁ receptors. As a result, these neurons via endocannabinoids can integrate signals from the periphery, such as those induced by nociceptive and stressful stimuli. In particular, in freely moving rats a peripheral noxious stimulation of the tail determined a TRPV₁-dependent increase in extracellular DA levels in the NAc [87]. Notably, no identification of the endogenous mediator(s) has been provided yet, and no direct measurements of endocannabinoids/endovanilloids have been performed under those conditions. However, the observations that activation of TRPV₁ in acute midbrain slice preparations increases the firing rate of VTA DA cells [87, 113], a mechanism dependent upon presynaptic facilitation of glutamatergic transmission, suggest the involvement of an endovanilloid/endocannabinoid molecule (e.g. anandamide, NADA) [87, 154]. A similar scenario occurs within the SNpc in DA cells [8, 112]. Remarkably, to date, different studies propose discrete lipid molecules as mediators of presynaptic facilitation of glutamatergic transmission in the VTA and SNpc. Hence, while in the VTA anandamide is in the spotlight for such a fine tuning of DA cell excitability via presynaptic inhibition or facilitation of glutamate release downstream of activation of CB₁ and TRPV₁ receptors [154], respectively, in the SNpc NADA appears to be acting in such a position [8].

Noteworthy, as mentioned above in this chapter, anatomical and functional heterogeneity of midbrain DA neurons has been well established [30, 41, 42, 155]. Accordingly, a subset of VTA DA neurons can decrease their impulse activity in response to a noxious stimulus, such as the tail-pinch, as a result from tVTA/RMTg activation [108, 111]. Consequently, given that the tVTA/RMTg nucleus strongly projects to the SNpc, and that tVTA/RMTg afferents onto VTA DA cells are under negative control of CB₁ receptors [93, 108, 156], one would expect these latter to be also located on tVTA/RMTg terminals onto SNpc cells. However, since different subsets of midbrain DA neurons receive topographic inputs from different sub-regions of the tVTA/RMTg [80], this prediction might not be correct.

Midbrain DA neurons also confer the individual with the capability to update and adapt to formerly learned behavioral responses in a changing environment, and this is essential for coping with adverse events. Notably, mesocortical DA transmission has long been involved in cognitive flexibility processes contributing to behavioral flexibility [157], which takes account of both set-shifting and reversal learning. Importantly, all of these phenomena are markedly impaired by stress [158–161]. In addition, converging evidence suggests the involvement of the endocannabinoid system in stress-induced responses [162–168]. Hence, genetic deletion of CB₁ receptors induces hypersensitivity to stress [162, 169], being rodents more vulnerable to stress-induced, depression-like changes in behavior and gene expression [162, 164, 168, 170–174]. Conversely, FAAH deficient mice display reduced anxiety-like behaviors in both the elevated plus maze and the light-dark box, both effects requiring CB₁ receptors activation [175, 176]. Remarkably, while endocannabinoid levels

are influenced by acute and chronic stressors in diverse brain regions [168, 177, 178], direct measurements within the VTA and SNpc have never been performed. However, in socially isolated rats changes in mRNA expression of DAGL isozyme β were found in both the VTA and SNpc [179]. Noteworthy, important excitatory inputs onto VTA DA cells undergo aberrant plasticity in response to stress in CB₁ receptor deficient mice [180], and this might ultimately influence VTA DA neuronal activity. In particular, the anterolateral portion of the BNST shows a widespread connectivity with those systems that are phylogenetically conserved to process stress signals [181]. Hence, activation of the PFC (in particular of the infralimbic portion) enhances excitatory afferents to the BNST, which in turn excites BNST neurons projecting to the VTA [76, 77], thus leading to their phasic activation [182]. In this scenario, CB₁ receptor activation within the BNST dampens VTA DA cell excitation induced by PFC stimulation, thus unveiling the role of endocannabinoids in this neural circuit [182, 183]. Therefore, the observation that in CB₁ receptor deficient mice acute stress shifts synaptic plasticity at excitatory afferents onto BNST neurons from LTD to LTP [180] was somehow predictable. Accordingly, pharmacological blockade of CB₁ receptors within the PFC is not able to reduce stress-evoked increases of extracellular DA levels in the PFC [184]. Nonetheless, caution should be used when comparing the aforementioned studies given that the procedures to induce acute stress are different, being acute restraint stress [180] and acute tail-pinch stress [184], respectively. Conversely, while one study reported that rat prefrontal glucocorticoid receptors play a role in stress-induced enhanced DA levels within the PFC [184], the other ruled out the involvement of mouse glucocorticoid receptor activation in the switch of CB₁ receptor-dependent synaptic plasticity at excitatory synapses onto BNST [180]. Irrespective of the diverse species and stressors used, however, it is worth to mention that acute restraint stress does increase both the firing rate and bursting pattern of VTA DA neurons in awake rats [185]. Whether or not the endocannabinoid system is involved in such an increased frequency and pattern of discharge of VTA DA neurons remains to be elucidated.

Concluding Remarks

The endocannabinoid system plays a fundamental role in making short- and long-term modifications to DA neural circuits and related behaviours. Nevertheless, many facets of this interplay are still unclear. For instance, it remains to elucidate whether or not there is a bias for one of the diverse endocannabinoids to be mobilized by DA cells *on demand*, or to be tonically produced depending on the state the synapse resides. Since CB₁ receptors are abundantly expressed in the midbrain, and both anandamide and NADA serve two masters (i.e. CB₁ and TRPV1 receptors) located on terminals impinging upon midbrain DA cells, it is tempting to speculate that neuromodulatory functions of the “extended family” of endocannabinoids might help keeping synaptic efficacy within those dynamic ranges that guarantee a proper integration of interoceptive stimuli and sensory information. Importantly, this balance is processed in order to facilitate motor control and promote learning.

The number of exciting discoveries brought up to the scientific community almost on a daily basis highlights the importance of such a fine regulation. Hence, given that deficits in DA neuromodulation contribute to the pathophysiology of several neuropsychiatric disorders, and the emerging and prominent role of the endocannabinoid system in modulating DA neuronal activity and transmission, pharmacotherapies aimed at precisely regulating the endogenous levels represent a promising treatment for diverse psychiatric and neurological disorders. Equally important is that potential therapeutic benefits of cannabis and cannabinoids are currently under heavy analysis in many countries worldwide.

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