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Guanfacine's mechanism of action in treating prefrontal cortical disorders: Successful translation across species

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ABSTRACT

The selective norepinephrine (NE) α2A-adrenoceptor (α2A-AR) agonist, guanfacine (Intuniv™), is FDA-approved for treating Attention Deficit Hyperactivity Disorder (ADHD) based on research in animals, a translational success story. Guanfacine is also widely used off-label in additional mental disorders that involve impaired functioning of the prefrontal cortex (PFC), including stress-related disorders such as substance abuse, schizotypic cognitive deficits, and traumatic brain injury. The PFC subserves high order cognitive and executive functions including working memory, abstract reasoning, insight and judgment, and top-down control of attention, action and emotion. These abilities arise from PFC microcircuits with extensive recurrent excitation through NMDAR synapses. There is powerful modulation of these synapses, where cAMP-PKA opening of nearby potassium (K^+) channels can rapidly and dynamically alter synaptic strength to coordinate arousal state with cognitive state, e.g. to take PFC "offline" during uncontrollable stress. A variety of evidence shows that guanfacine acts within the PFC via post-synaptic α2A-AR on dendritic spines to inhibit cAMP-PKA-K^+ channel signaling, thus strengthening network connectivity, enhancing PFC neuronal firing, and improving PFC cognitive functions. Although guanfacine's beneficial effects are present in rodent, they are especially evident in primates, where the PFC greatly expands and differentiates. In addition to therapeutic actions in PFC, stress-related disorders may also benefit from additional α2-AR actions, such as weakening plasticity in the amygdala, reducing NE release, and anti-inflammatory actions by deactivating microglia. Altogether, these NE α2-AR actions optimize top-down control by PFC networks, which may explain guanfacine's benefits in a variety of mental disorders.

1. Introduction

The norepinephrine (NE) α2A-adrenoceptor (α2A-AR) agonist, guanfacine, was approved by the FDA for the treatment of Attention Deficit Hyperactivity Disorder (ADHD) in 2009 under the brand name, Intuniv™, one of the rare success stories where basic neuroscience research in animals has successfully translated to human patients. The beneficial effects of α2-AR agonists for higher cognitive function were first discovered in aged monkeys (Arnsten, Cai, & Goldman-Rakic, 1988; Arnsten & Goldman-Rakic, 1985), who naturally develop cognitive impairments on tasks dependent on the prefrontal cortex (PFC), a newly evolved brain region that subserves working memory, abstract reasoning, and the top down regulation of attention, action and emotion (Szczepanski & Knight, 2014). Although α2-ARs are classically considered as presynaptic receptors, early research determined that the beneficial effects of α2-AR agonists on cognition arose from *postsynaptic* receptor actions in the PFC (Arnsten & Goldman-Rakic, 1985; Cai, Ma, Xu, & Hu, 1993), with a pharmacological profile consistent with the α2A-AR subtype (Arnsten et al., 1988; Arnsten & Leslie, 1991), a finding later confirmed in genetically altered mice (Franowicz et al., 2002). Subsequent research determined the cellular basis for guanfacine's beneficial actions, strengthening network connections in PFC through intracellular signaling events in dendritic spines (Wang et al., 2007). Guanfacine is now in widespread clinical use, not only in ADHD, but in additional disorders associated with impaired PFC function. The following review describes guanfacine's mechanism of action in PFC, enhancing the network connections needed for healthy cognitive experience and top-down control.

It is noteworthy that many of the discoveries made in this field were by women neuroscientists. As this recognition may provide inspiration to young women and girls wanting to become scientists, many of their names will be emphasized in this review.

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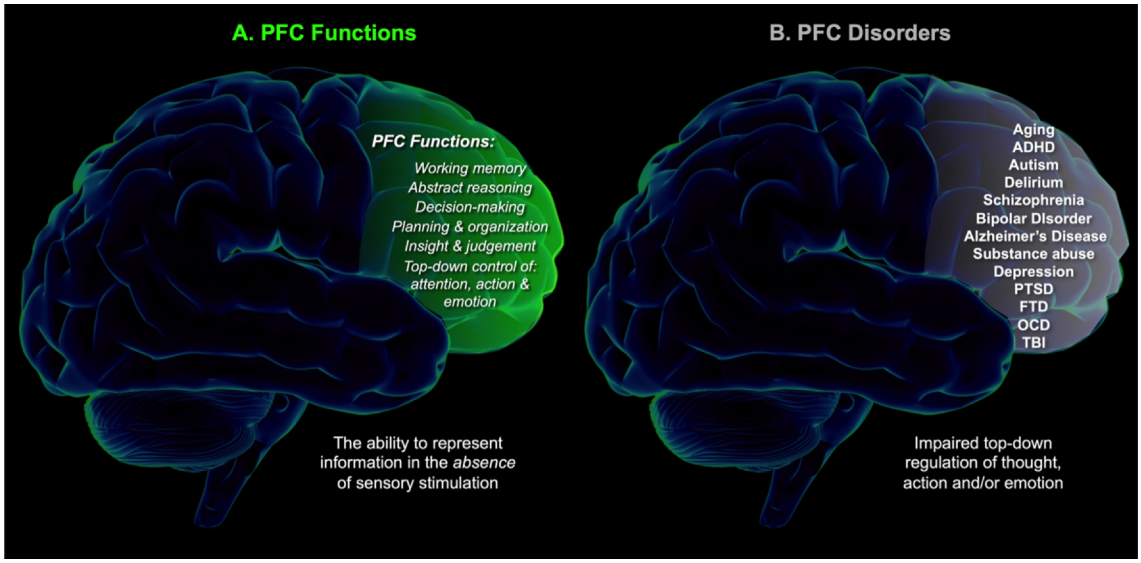

Fig. 1. (A) Schematic illustrations of the higher cognitive functions of the prefrontal cortex (PFC). (B) A list of disorders with impaired PFC functioning.

2. The cognitive functions of PFC and their dysfunction in mental disorders

2.1. Cognitive functions of the PFC

The newly evolved PFC subserves many of our highest order cognitive functions, representing, evaluating and manipulating information in the absence of sensory stimulation, and using that information for goal-directed behavior (Fuster, 2008). In general, the PFC in primates is topographically organized, with circuits in the dorsal and lateral PFC (dlPFC) representing information from the external world (e.g. through vision and audition), and the ventral and medial subregions representing the internal world (e.g. taste and smell, pain, reward and punishment) (Ongür & Price, 2000). The ability to generate mental representations underlies a range of cognitive operations', including working memory', high-order decision-making', insight and judgment', and the top-down regulation of attention', action and emotion (summarized in Fig. 1A). Many of the highest order abilities', i.e. *meta*-cognitive abilities ("thinking about thinking"', "remembering to remember"', and insight and judgment)', reside in the frontal pole', which expands greatly in human vs. nonhuman primates (Tsujimoto, Genovesio, & Wise, 2011). The human PFC also shows evidence of laterality, with the left hemisphere specialized for language production, and the right hemisphere especially important for behavioral inhibition, i.e. inhibiting inappropriate responses.

2.2. Mental disorders involving the PFC

PFC functions are impaired in a host of mental disorders (summarized in Fig. 1B). Some of these disorders arise from developmental insults and are evident early in life. For example, ADHD is associated with slowed (Shaw et al., 2007) or impaired (Shaw et al., 2009) development of the right lateral PFC, the PFC region needed for inhibiting inappropriate actions and attention to distractors (Aron, Robbins, & Poldrack, 2004). Autism spectrum disorders can afflict a variety of circuits with a range of severity, but share in common alterations in the circuits mediating social cognition, many of which reside in PFC (Shamay-Tsoory & Aharon-Peretz, 2007). Mood disorders such as depression are associated with changes in the medial PFC, including overactivation of the cingulate circuits that process the suffering aspects of pain (Mayberg et al., 2005; Opler, Opler, & Arnsten, 2016). In contrast, Blumberg has shown that these circuits are underactive in the manic phase of bipolar disorder, as are the areas affecting impulse control and insight (Blumberg et al., 2003, 1999), helping to explain why these disorders can sometimes look so similar in children. Schizophrenia is associated with accelerated gray matter loss in PFC (Cannon, Chung, He, Sun, Jacobson, van Erp, & Consortium, 2014), including loss of dendrites and spines in the dlPFC (Glantz & Lewis, 2000). Indeed, hypofrontality during working memory correlates with symptoms of thought disorder in patients with schizophrenia (Perlstein, Carter, Noll, & Cohen, 2001), a symptom that is worsened by stress exposure (Docherty, Evans, Sledge, Seibyl, & Krystal, 1994). As described below, stress exposure weakens PFC function, and severe stressors that cause PTSD can cause atrophy of PFC (Kühn & Gallinat, 2013; Meng & iang, Jin, Liu, Zhao, Wang, Gong, 2016). Stress exposure is also often a cause of substance abuse, which in turn further weakens PFC abilities, creating a vicious cycle (Sinha, 2007). Finally, aging causes weakening and removal of dlPFC synapses on spines (Morrison & Baxter, 2012), a process which is worsened by Alzheimer's Disease (AD), where tau pathology often begins in the medial and ventral areas and moves to dlPFC as the disease progresses, correlating with the rise in dementia (Bussière et al., 2003). If you are interested in learning more about the role of the PFC in mental disorders, the effects of stress on the PFC, or the neurobiology of AD, please access the following educational YouTube videos at the Yale School of Medicine YouTube channel:

The PFC and mental disorders: https://www.youtube.com/watch?v = DEtnoiKGDwI
The PFC and stress: https://www.youtube.com/watch?v = TsQUeNuvIDY
The neurobiology of Alzheimer's Disease: https://youtu.be/wa3dWrszpEQ

3. The neuronal basis of higher cognition in PFC

Much of our understanding of the cellular neurobiology of dlPFC function is due to the pioneering research of Patricia Goldman-Rakic, whose anatomical and physiological studies uncovered the cellular basis for working memory and abstract thought (Goldman-Rakic, 1995). Her early lesion studies determined the subregion of the dlPFC surrounding the principal sulcus that was essential for visospatial working memory. Physiological recordings from this area, in concert with neuroanatomical mapping, revealed how dlPFC microcircuits, connected with multiple cortical and subcortical networks, can generate the mental representations needed for thoughtful behavior.

3.1. Cortical networks-

Goldman-Rakic's anatomical tracing studies (Goldman-Rakic, 1987; Selemon & Goldman-Rakic, 1988), along with the elegant work of many other women neuroscientists (e.g. Ann Graybiel, Lynn Selemon, Helen Barbas, Suzanne Haber), revealed the extensive PFC connections with both cortical and subcortical brain regions. These studies have shown that PFC circuits allow the top-down regulation of sensory association cortices e.g. for control of attention (Barbas et al., 2005), and of subcortical structures such as the amygdala for control of emotion (Ghashghaei, Hilgetag, & Barbas, 2007), and with the basal ganglia, such as the projections to the caudate and subthalamic nucleus, e.g. for the inhibition of inappropriate responses (Graybiel, 1991; Haber, 2016; Haynes & Haber, 2013; Selemon & Goldman-Rakic, 1985).

3.2. Delay cell physiology

The early work of Fuster found neurons in the dlPFC that were able to maintain firing across the delay period in a working memory task, e.g. for 18 sec (Fuster & Alexander, 1971). Goldman-Rakic and colleagues (Funahashi, Bruce, & Goldman-Rakic, 1989) built on this original discovery using an oculomotor visuospatial working memory task (Fig. 2A). They found dlPFC "Delay cells" can represent a position in visual space across the delay period when there is no sensory stimulation through spatially-tuned, persistent firing for their preferred spatial direction, but not for other, nonpreferred locations (Fig. 2B). These Delay cells can maintain firing even through distractions (e.g. the work of Jacqueline Gottlieb (Suzuki & Gottlieb, 2013)). The ability to generate mental representations is fundamental to abstract reasoning and top down control, e.g. inhibiting inappropriate behavior (Funahashi, Chafee, & Goldman-Rakic, 1993), and regulation of attention (Buschman & Miller, 2007).

3.3. Layer III microcircuits

Goldman-Rakic also discovered the dlPFC microcircuits that generate mental representations, concentrated in deep layer III of the dlPFC (Fig. 2C), a layer that expands greatly in primates. With Mary Kritzer, she showed that deep layer III contains the extensive horizontal connections needed for extensive recurrent excitation (Kritzer & Goldman-

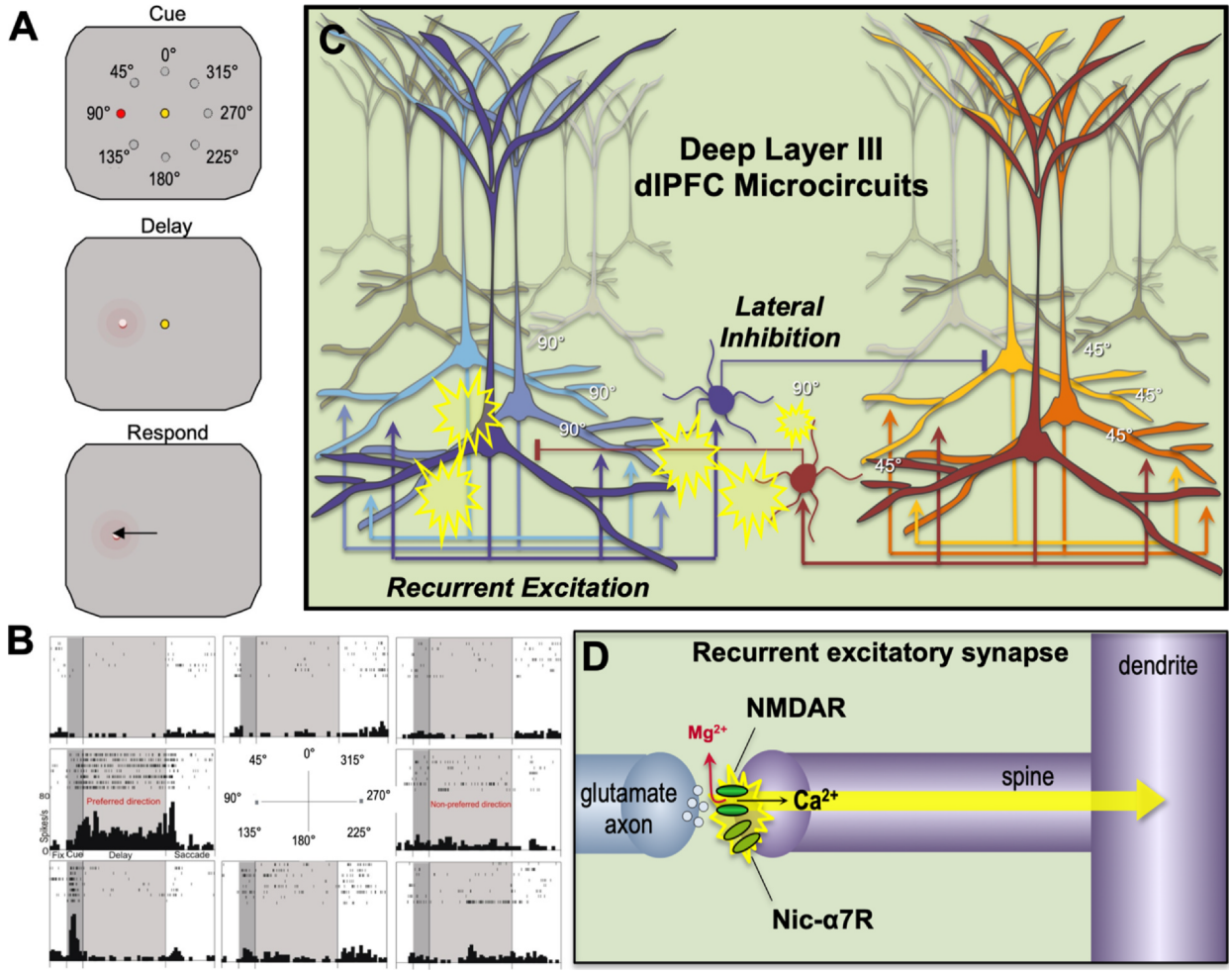

Fig. 2. Spatial working memory circuits of the dlPFC. (A) The oculomotor delayed response (ODR) test of spatial working memory often used to probe the physiological functioning of the dlPFC in monkeys. (B) An example of a dlPFC Delay cell with spatially tuned, persistent firing across the delay period for the neuron's preferred direction (90°) but little firing for other spatial positions. (C) The microcircuits in deep layer III dlPFC that are thought to underlie spatial working memory. Persistent firing arises from extensive recurrent excitation, where pyramidal cells with shared preferred directions excite each other to keep information "in mind" over the delay period. Spatial tuning is refined by lateral inhibition from parvalbumin-containing GABAergic interneurons, i.e. basket and chandelier cells. (D) Recurrent excitation on spines depends on glutamate stimulation of NMDAR, with surprisingly little contribution from AMPAR. Instead, the permissive effects of AMPAR to depolarize the synaptic membrane and eject magnesium from the NMDAR pore appear to be performed by acetylcholine, including nic-α7R that reside within and near the glutamate synapse. As acetylcholine is released according to arousal state, effective neurotransmission in the dlPFC depends on arousal conditions.

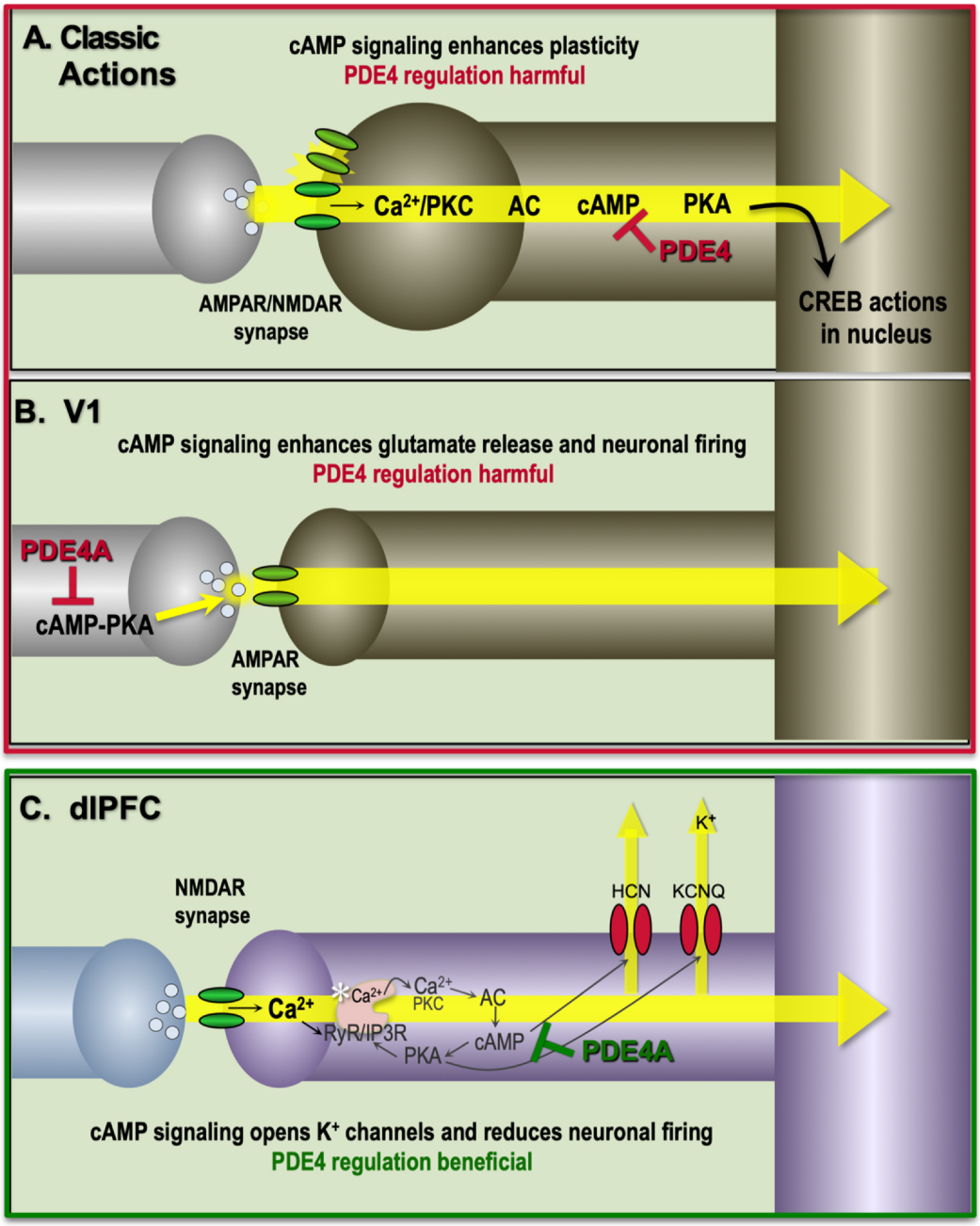

Fig. 3. cAMP-PKA actions in classic circuits vs. dlPFC. (A) In classic circuits, cAMP-PKA signaling in spines enhances plasticity, e.g. via activation of CREB, and PDE4 inhibition improves learning and memory. (B) cAMP-PKA signaling also has classic actions at pre-synaptic sites, e.g. enhancing glutamate release, as occurs in monkey primary visual cortex V1. C. In layer III dlPFC spines, cAMP-PKA signaling opens nearby K^+ channels (e.g. KCNQ2) to *reduce* firing. Thus, PDE4 inhibition can be harmful in these circuits.

Rakic, 1995). She posited that the persistent firing across the delay period arose from pyramidal cells with shared "preferred directions", exciting each other through glutamatergic synapses on spines (Fig. 2C-D). Thus, one needs a large number of strong, excitatory connections to maintain the firing across a long delay period. Conversely, she showed evidence that the spatial tuning was refined by lateral inhibition from GABAergic interneurons, whereby pyramidal cells could inhibit their neighbors with dissimilar preferred directions to enhance "signal to noise" (Fig. 2C). This working model was confirmed by extraordinary *in vitro* studies by Gonzalez-Burgos, identifying the neurons involved in recurrent excitation and lateral inhibition (González-Burgos, Barrionuevo, & Lewis, 2000; González-Burgos, Krimer, Povysheva, Barrionuevo, & Lewis, 2005).

4. Unique molecular regulation of layer PFC microcircuits

Layer III dlPFC microcircuits that generate mental representations have unique molecular needs for both neurotransmission and neuromodulation that allow them to sustain long periods of neuronal firing without bottom-up sensory stimulation (Arnsten, 2015; Arnsten, Wang, & Paspalas, 2012). However, these same molecular signaling requirements confer vulnerability to dysfunction when not properly regulated due to genetic and/or environmental insults.

4.1. *Unique neurotransmission-*

Classic glutamate neurotransmission depends on stimulation of

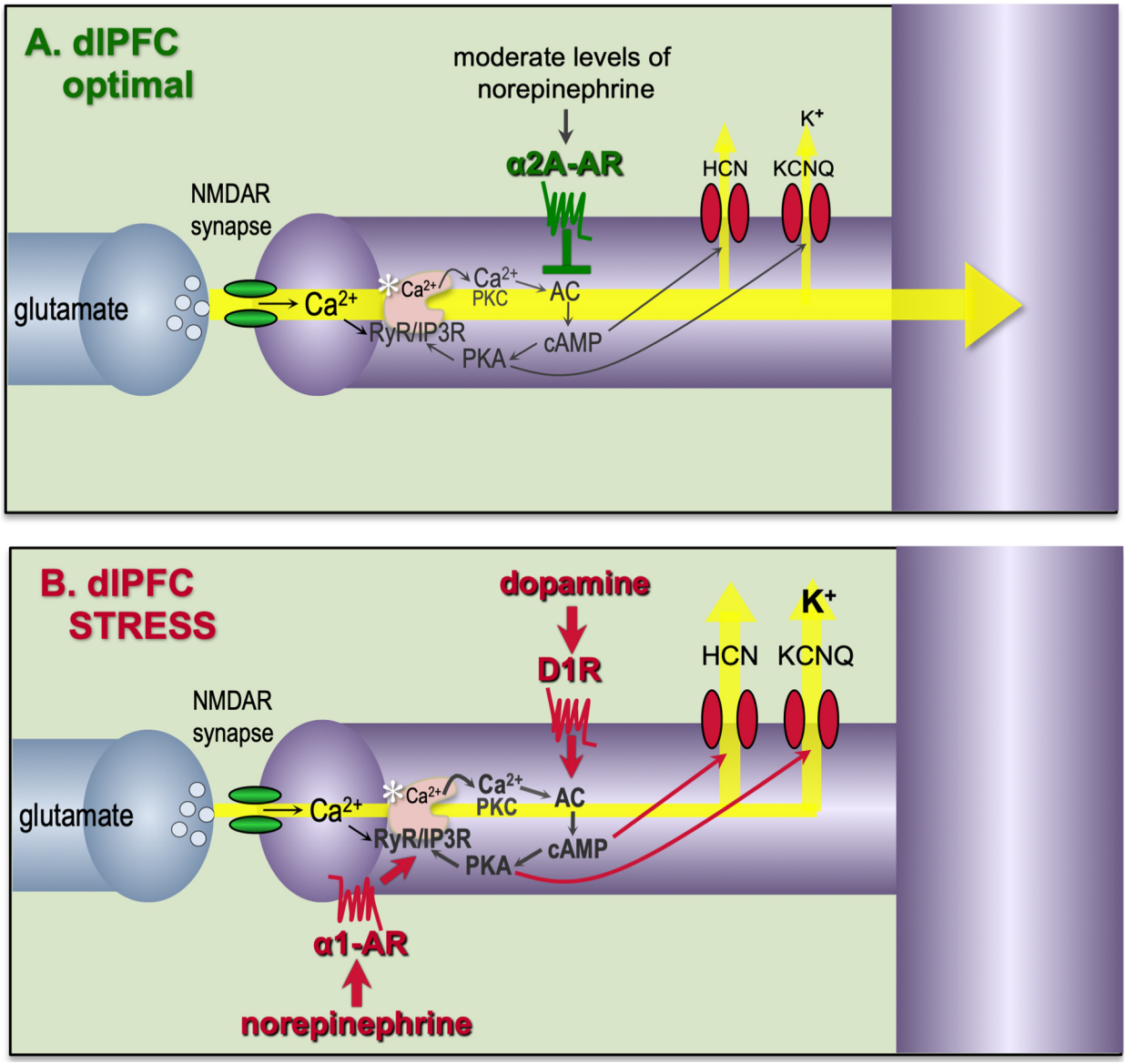

Fig. 4. Catecholamine actions in dlPFC during optimal vs. stressful arousal conditions. (A) Under optimal arousal conditions (safe, alert, interested), there are moderate levels of NE release that engage high affinity α2A-AR which inhibit feedforward, Ca^{2+}-cAMP-PKA-K^+ signaling to strengthen connectivity and enhance neuronal firing. (B) Under conditions of uncontrollable stress, high levels of catecholamines are released in PFC. High levels of NE engage lower affinity α1-AR, and high levels of dopamine release engage D1R, both of which drive feedforward Ca^{2+}-cAMP-PKA-K^+ signaling to weaken connectivity and reduce neuronal firing.

AMPAR to depolarize the membrane, to eject the magnesium (Mg^{2+}) block from the NMDAR pore, and permit NMDAR transmission. Neurons in primary visual cortex (V1) of the rhesus monkey exhibit classic neurotransmission, relying heavily on AMPAR neurotransmission, with only a minor NMDAR component (Yang et al., 2018). The rapid kinetics of AMPAR are appropriate for these circuits, whose function is to rapidly and accurately encode the appearance/disappearance of visual events in the environment. In contrast, the work of Min Wang and colleagues (Wang et al., 2013) have shown that Delay cells in the dlPFC have remarkably little reliance on AMPAR, and instead rely heavily on NMDAR, including NMDAR with NR2B subunits, that close slowly and transfer large amounts of calcium (Ca^{2+}) into the spine (Fig. 2D). This experimental finding was predicted by computational models (Wang, 1999). Wang went on to show that the permissive role of AMPAR in classic synapses is instead played by acetylcholine in dlPFC Delay cells. Her work with Yang Yang showed that cholinergic stimulation of nic-α7R, which are localized within the glutamate PSD in layer III dlPFC, are permissive for NMDAR stimulation (Fig. 2D; (Yang et al., 2013)). Nic-α7R are particularly effective in fluxing Ca^{2+} (Fucile, 2004), which may help to sustain membrane depolarization needed for continuous firing. A more recent study by Wang and Galvin showed that muscarinic M1R also permits NMDAR Delay cell firing, possible exciting the synaptic membrane via closure of synaptic KCNQ "m" channels, and/or by increasing internal Ca^{2+} release via Gq-IP3 signaling (Galvin et al., 2020). As acetylcholine is released during waking but not deep sleep, this unusual form of synaptic transmission may explain why dlPFC microcircuits can communicate during waking to allow conscious mental activity, but not during deep sleep when we are unconscious.

4.2. Unique neuromodulation

Layer III dlPFC microcircuits also have different neuromodulatory needs than typical circuits, where neuromodulators further coordinate cognitive state with arousal state, including rapidly disconnecting dlPFC circuits during uncontrollable stress (Arnsten, 2015; Arnsten

et al., 2012). In classic circuits, cAMP signaling strengthens synaptic actions, e.g. by enhancing plasticity in hippocampal circuits, including activation of transcriptional events for very long-term changes in synaptic strength (Fig. 3A; (Abel et al., 1997; MacKenzie & Houslay, 2000)), or by presynaptic facilitation of glutamate release, as occurs in V1 where cAMP signaling increases neuronal firing (Fig. 3B; (Yang et al., 2018)). Thus, in hippocampus, phosphodiesterases such as PDE4A impair memory consolidation, and PDE4 inhibitors improve long-term memory storage (eg (Havekes et al., 2016; Wimmer, Blackwell, & Abel, 2020)).

In contrast to these classic circuits, the working memory circuits of the dlPFC are modulated very differently, consistent with their role in creating the more ephemeral, constantly-changing representations required for effective working memory. In these circuits, cAMP-PKA signaling opens nearby potassium (K^+) channels to rapidly *weaken* synaptic strength and reduce Delay cell firing (Fig. 3C; (Arnsten et al., 2012)). Early studies with Jane Taylor showed that activation of cAMP-PKA signaling in rat mPFC impaired working memory performance (Taylor, Birnbaum, Ubriani, & Arnsten, 1999), opposite to its enhancing effects on long-term memory consolidation in hippocampus and amygdala. Ultrastructural analysis of the primate layer III dlPFC by Paspalas and colleagues showed a constellation of cAMP-related proteins near the Ca^{2+}-containing spine apparatus (the extension of the smooth endoplasmic reticulum into the spine), near HCN and KCNQ channels, providing structural evidence for feedforward Ca^{2+}-cAMP-K^+ channel signaling in layer III dlPFC spines (Carlyle et al., 2014; Galvin et al., 2020; Paspalas, Wang, & Arnsten, 2013; Wang et al., 2007). Thus, rapid changes in cAMP-Ca^{2+}-K^+ channel signaling can produce dynamic changes in synaptic strength to coordinate environmental events and arousal with cognitive state, a process termed Dynamic Network Connectivity (Arnsten et al., 2012). Under optimal conditions (alert, safe, interested), moderate levels of NE release engage high affinity α2A-AR that inhibit cAMP-K^+ channel signaling to strengthen synaptic connectivity (Fig. 4A; (Wang et al., 2007)). Similar effects have now been seen with post-synaptic mGluR3 signaling (Jin et al., 2018), a receptor consistently linked to risk of schizophrenia (Saini et al., 2017), and more recently, human intelligence (Zink et al., 2020). Thus, strong layer III recurrent connections are important for strong cognition. However, under conditions of uncontrollable stress, there are high levels of NE and DA release in the PFC (Finlay, Zigmond, & Abercrombie, 1995; Murphy, Arnsten, Goldman-Rakic, & Roth, 1996), which engage α1-AR and D1R to drive Ca^{2+}-PKC and cAMP-PKA signaling, rapidly opening nearby K^+ channels to weaken PFC network connectivity and impair working memory and top-down control (Fig. 4B); (Arnsten et al., 2019; Birnbaum et al., 2004; Gamo et al., 2015). Thus, feedforward Ca^{2+}-PKC-cAMP-PKA-K^+ signaling can very quickly take PFC "offline".

The amygdala responds to NE in an opposite manner to the mPFC, thus producing a chemical "flip-flop" switch to determine which structure is positioned to orchestrate the brain's response. The engagement of α2A-AR by moderate levels of NE release under safe conditions weakens the amygdala (DeBock et al., 2003), while high levels of catecholamine release during stress strengthen its emotional responses through α1-AR and β-AR (Debiec & LeDoux, 2006; Ferry, Roozendaal, & McGaugh, 1999). Thus, under nonstress conditions of basal NE release, high affinity α2A-AR strengthen the top-down orchestration by the PFC and weaken the amygdala, while under conditions of stress, high levels of NE release engage low affinity α1-AR and β-AR to weaken the PFC and strengthen the amygdala. This rapid switch may be beneficial under some conditions, e.g. being cut off on the highway, but can be devastating when dealing with a stressor that requires PFC abstract reasoning, e.g. an invisible virus such as COVID-19.

4.3. The effects of chronic stress exposure

With chronic stress exposure, there is architectural loss of mPFC spines and dendrites (Moda-Sava et al., 2019; Radley et al., 2006) that correlates with impaired PFC cognitive functioning (Hains et al., 2009; Hains, Yabe, & Arnsten, 2015; Liston et al., 2006). In contrast, the dendrites of amygdala neurons actually expand with chronic stress exposure (Vyas, Mitra, Shankaranarayana Rao, & Chattarji, 2002), as do the dendrites of the subset of neurons in PFC that excite the amygdala (Shansky, Hamo, Hof, McEwen, & Morrison, 2009), while cortical-cortical neurons in mPFC lose their dendrites (Shansky et al., 2009). In this way, the switch from thoughtful to reactive network connectivity is codified by architectural changes. Reduced PFC gray matter and weaker functional connectivity with chronic has been seen in humans as well, (Ansell, Rando, Tuit, Guarnaccia, & Sinha, 2012; Liston, McEwen, & Casey, 2009). These changes can be reversed in both rats and humans with extended periods of nonstress, at least in young subjects (Bloss et al., 2011; Liston et al., 2009). The molecular basis for spine loss is particularly interesting, given its relevance to many mental disorders. Research in rats by Avis Hains has shown that inhibition of PKC or PKA signaling can prevent the loss of mPFC dendritic spines and working memory abilities during chronic stress exposure (Hains et al., 2009, 2015), suggesting that the sustained weakening of synaptic connectivity via K^+ channel opening contributes to spine loss. These data also have immediate relevance to treatment of mental disorders, as agents such as lithium and atypical antipsychotics reduce PKC signaling (Manji & Lenox, 1999), and daily guanfacine administration prevented spine loss and working memory deficits (Hains et al., 2015), which may help to explain its utility in treating stress-related disorders.

Evidence suggests that physiological stressors such as traumatic brain injury (Kobori, Clifton, & Dash, 2006; Kobori, Hu, & Dash, 2011; Kobori, Moore, & Dash, 2015), and hypoxia (Kauser, Sahu, Kumar, & Panjwani, 2013) have similar physiological actions as psychological stress within the PFC. These physical stressors also induce catecholamine release in mPFC, and activate the same intracellular signaling events (e.g. activation of cAMP-PKA signaling), in association with dendritic spine loss and working memory impairment. Thus, multiple starting points can lead to the same phenotype of impaired PFC structure and function.

It is noteworthy that there are marked sex differences in the stress response, where female animals and humans have a greater stress response (Shansky et al., 2004) and humans (Qin et al., 2012), which may help to explain the greater prevalence of PTSD and depression in women, e.g. as shown by Weissman and Mazure (Breslau, Chilcoat, Kessler, Peterson, & Lucia, 1999; Maciejewski, Prigerson, & Mazure, 2001; Weissman et al., 1996). Research in rodents has begun to illuminate the biological factors that underlie these sex differences. Rita Valentino has found that the extended amygdala produces greater activation of the NE locus coeruleus in females (Bangasser & Valentino, 2012), while Rebecca Shansky has shown a range of sex-specific differences in the stress response (Rincón-Cortés, Herman, Lupien, Maguire, & Shansky, 2019), including exaggerated dendritic changes in the PFC of estrogen-exposed females (Shansky et al., 2010). Estrogen also reduces the expression of COMT (Tunbridge, 2010), one of the catabolic enzymes that breaks down catecholamines, which would thus exacerbate the stress response in women. Recent data also indicate that the increased risk of stress-induced depression in females is associated with long non-coding RNAs that regulate CREB signaling in the PFC (Issler et al., 2020). As discussed below, these factors may help to explain why guanfacine is particularly helpful in treating women with stress-related disorders such as substance abuse.

5. Guanfacine's mechanism of action in PFC

A variety of evidence indicates that guanfacine improves PFC function by mimicking NE's beneficial actions at post-synaptic α2A-AR

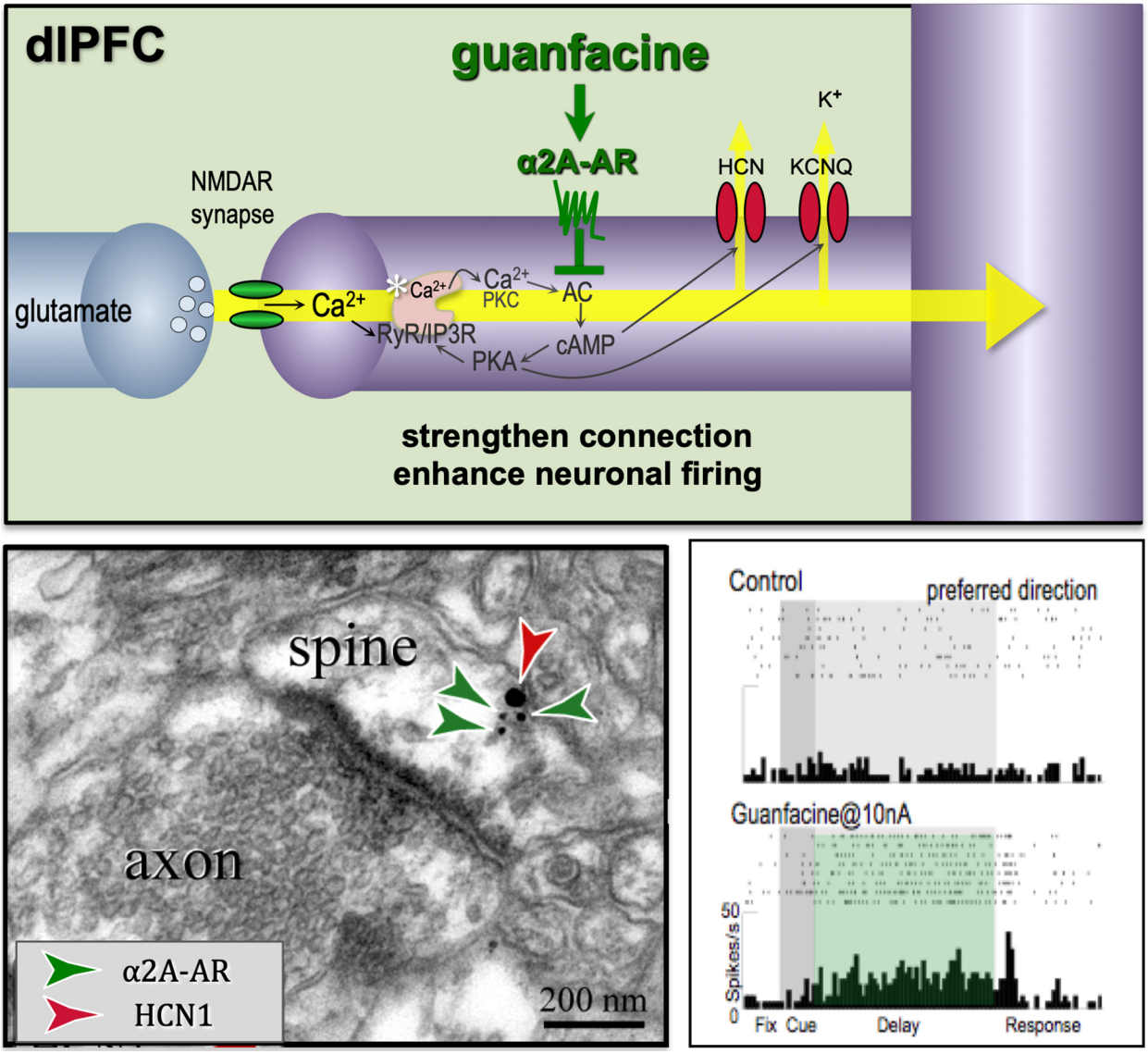

Fig. 5. Guanfacine's mechanism of action in the primate dlPFC. (A) A schematic drawing showing how guanfacine stimulation of α2A-AR on dlPFC dendritic spines inhibits cAMP-PKA-K^+ signaling to strengthen connectivity and thus enhance neuronal firing. Note that emerging data suggest that HCN channels on PFC spines may open neighboring K^+ channels (El Hassar, Arnsten, Datta and Kaczmarek, unpublished), and thus behave as an ion channel complex. (B) ImmunoEM shows that α2A-AR are co-localized with HCN channels on layer III dlPFC spines. Adapted from (Wang et al., 2007) with permission; image originally created by C. Paspalas. (C) Iontophoresis of guanfacine onto a dlPFC Delay cell enhances task-related firing (highlit in green). Adapted from (Wang et al., 2007) with permission; image originally created by M. Wang. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

on dlPFC spines, strengthening PFC network connectivity via inhibition of cAMP-PKA-K^+ signaling (Fig. 5A). This mechanism of action has been clarified by decades of research in animals and humans.

5.1. Actions at post-synaptic receptors of the α2A-AR subtype

Early studies discovered that α2-AR agonists markedly improved working memory performance in young monkeys with experimental catecholamine depletion in the dlPFC, with effects consistent with actions at post-synaptic receptors in the dlPFC (Arnsten & Goldman-Rakic, 1985). α2-AR agonists also improved working memory performance in aged rhesus monkeys with naturally occurring catecholamine depletion (Arnsten et al., 1988; Arnsten & Goldman-Rakic, 1985; Arnsten & Leslie, 1991; Rama, Linnankoski, Tanila, Pertovaara, & Carlson, 1996). It is noteworthy that cAMP signaling becomes dysregulated in the aging PFC due to loss of PDE4 expression (Carlyle et al., 2014), and that regulation through α2A-AR is especially helpful in restoring PFC neuronal firing (Wang et al., 2011). Thus, the use of aged animals likely contributed to the prominence of this initial discovery. Research with visiting Chinese scientist, Jing-Xia Cai, showed that the α2A-AR-selective agonist, guanfacine, was the most potent agent with the least side effects, suggesting that the beneficial effects arose from what is now called the α2A-AR subtype (Arnsten et al., 1988). This hypothesis was later confirmed in mice with genetic mutations to the α2A-AR (Franowicz et al., 2002). Dr. Cai returned to China and taught generations of scientists about the PFC, thus establishing a global collaboration that continues to this day.

5.2. Actions directly in the PFC

At least some of guanfacine's beneficial actions on cognition result from actions within the PFC. For example, direct infusions of guanfacine into the lateral PFC improved performance of working memory and associative learning tasks in monkeys (Mao, Arnsten, & Li, 1999;

Wang, Tang, & Li, 2004). Guanfacine infusions into the aged rat mPFC also improve working memory, and are reversed by local activation of cAMP-PKA signaling (Ramos, Stark, Verduzco, van Dyck, & Arnsten, 2006). Conversely, infusion of the α2-AR antagonist, yohimbine, into the monkey dlPFC impaired working memory (Li & Mei, 1994), impulse control (Ma, Qi, Peng, & Li, 2003) and regulation of locomotor activity (Ma, Arnsten, & Li, 2005), producing an "ADHD-like" phenotype.

5.3. Actions at the cellular level, inhibiting cAMP opening of K^+ channels on spines

Guanfacine and yohimbine also produce markedly opposing effects on the neuronal firing of dlPFC Delay cells in monkeys performing the ODR working memory task. Local iontophoresis of yohimbine directly onto dlPFC neurons markedly reduced their firing (Li, Mao, Wang, & Mei, 1999; Wang et al., 2007), while iontophoresis of guanfacine enhanced neuronal firing (Fig. 5C; (Wang et al., 2007)). It is noteworthy that there could be ceiling effects; i.e. neurons with strong Delay firing under basal conditions showed less effect of guanfacine, which may help to explain why guanfacine has minimal effects in healthy subjects with strong cognition. The physiological data also showed that α2A-AR stimulation enhances Delay cell firing by inhibiting cAMP-PKA opening of HCN channels. The enhancing effects of guanfacine were reversed by co-activation of cAMP-PKA signaling, while the loss of firing with yohimbine was reversed by HCN channel blockade (Wang et al., 2007).

ImmunoEM demonstrated the physical substrate for these physiological interactions, showing that HCN channels are co-localized with α2A-AR on dlPFC dendritic spines, e.g. on the spine head (Fig. 5B) and in the spine neck. Later studies showed that KCNQ2 channels, which are opened by PKA signaling, are also localized on spines near the synapse and the spine apparatus (Arnsten et al., 2019). Thus, the constellation of signaling molecules needed to dynamically alter network strength are all concentrated in layer III dlPFC dendritic spines, supporting the model proposed in Fig. 5. Computational models are also consistent with the hypothesis (Duggins, Stewart, Choo, & Eliasmith, 2017; Hassani et al., 2017). Thus, support comes from a range of approaches and perspectives.

5.4. Guanfacine protects PFC neurons from psychological and physiological stress

Evidence from both *in vivo* and *in vitro* studies show that guanfacine can protect PFC circuits from stress exposure, including preventing atrophy due to chronic stress exposure. This has been seen for both psychological stressors such as restraint stress in rodents (Hains et al., 2015), as well as for physiological stressors such as reduced oxygen (Kauser et al., 2013). As both psychological and physiological stressors drive feedforward calcium-cAMP-PKA-K^+ signaling and impair working memory, guanfacine's ability to inhibit these actions may preserve network connectivity and PFC function (schematically shown in Fig. 6A).

The work of Shari Birnbaum showed that pretreatment with guanfacine protects working memory from acute stress exposure in rats (Birnbaum, Podell, & Arnsten, 2000). Interestingly, guanfacine was more effective than clonidine (Birnbaum et al., 2000), suggesting that these benefits are not primarily due to reducing NE release, as clonidine is more potent than guanfacine in this regard. With chronic stress exposure, dendritic spines are lost from layer II/III pyramidal cells in rat mPFC (Fig. 6B-C; (Hains et al., 2009, 2015)). The work of Avis Hains showed that daily treatment with guanfacine prevented stress-induced loss of spines (Fig. 6C), and preserved working memory performance (Hains et al., 2015) These data may have relevance to guanfacine's therapeutic clinical effects in stress-related disorders.

Guanfacine also protects mPFC dendritic spines, neurons and working memory performance from the physiological stress of hypoxia (Kauser et al., 2013). Guanfacine's prevention of neuronal loss was associated with increased BDNF and decreased expression of caspase 3 (Kauser, Sahu, & Panjwani, 2016), which may be induced by calcium overload of mitochondria with high levels of cAMP-calcium signaling (Lencesova & Krizanova, 2012). These *in vivo* data are consistent with *in vitro* experiments showing that guanfacine enhances the maturation of dendritic spines in PFC neuronal cultures (Ren, Liu, & Li, 2011).

In addition to direct actions on dendritic spines, guanfacine's protective effects may involve anti-inflammatory actions through α2A-AR on glia (Gyoneva & Traynelis, 2013). As schematized in Fig. 6A, α2A-AR are expressed on activated microglia, where their engagement deactivates microglial activity (Gyoneva & Traynelis, 2013). Thus, guanfacine may reduce microglial phagocytosis of spines. The anti-inflammatory effects of α2A-AR agonists are well-established, and contribute to their widespread use as co-anesthetics in surgery, where they reduce the incidence of emergence delirium (Zhang et al., 2020). These anti-inflammatory effects may also contribute to guanfacine's protections from hypoxia in animal models (Kauser et al., 2013, 2016), and from encephalitis in humans (Singh-Curry, Malhotra, Farmer, & Husain, 2011). Thus, guanfacine's engagement of α2A-AR in PFC may have coordinated actions, both strengthening connections on spines and reducing local phagocytic actions by glia.

6. Guanfacine improves PFC-dependent cognition across species

Systemic administration of guanfacine improves PFC cognitive functions in rodents, monkeys and humans, a rare instance where a mechanism can be bridged across species. As the PFC expands greatly in primates (Wise, 2008), these actions are particularly important in these higher species, and may be why α2A-AR agonist pro-cognitive effects were initially discovered in monkeys. For example, rodents do not have a dlPFC or frontal pole, and even the medial aspects of PFC have recently been found to have very differing organizations (Wallis, Cardinal, Alexander, Roberts, & Clarke, 2017). Emerging data also indicate primate-unique cell types, and profound differences in transcriptomic signatures between rodents and primates (Krienen et al., 2019). Thus, translational research for cognitive disorders should try to use primates whenever possible.

6.1. Mice

Systemic administration of guanfacine significantly improves working memory performance in wild-type mice performing the delayed alternation task (Fig. 7A; (Franowicz et al., 2002)). Importantly, guanfacine does not improve mice with a genetic mutation of the α2A-AR where the receptor is no longer able to bind agonists, confirming actions at the α2A-AR subtype (Franowicz et al., 2002).

6.2. Rats

Guanfacine also improves working memory performance on the delayed alternation task in rats. Working memory performance was improved by systemic administration of guanfacine in stressed rats (Birnbaum et al., 2000), and by direct infusion into the mPFC of aged rats, with benefits reversed by the cAMP-PKA activator, Sp-cAMPS (Fig. 7B; (Ramos et al., 2006)). Systemic administration of guanfacine also improves attentional performance in a rat model of ADHD, Spontaneously Hypertensive rats (Kawaura, Karasawa, Chaki, & Hikichi, 2014; Sagvolden, 2006), that have altered catecholamines in mPFC (Russell, 2002).

6.3. Monkeys

Systemic administration of guanfacine in rhesus macaques improves a variety of cognitive functions dependent on the PFC. Guanfacine administration to aged monkeys improves working memory (Arnsten et al., 1988) and reduces disruption by distractors (Fig. 7C; (Arnsten &

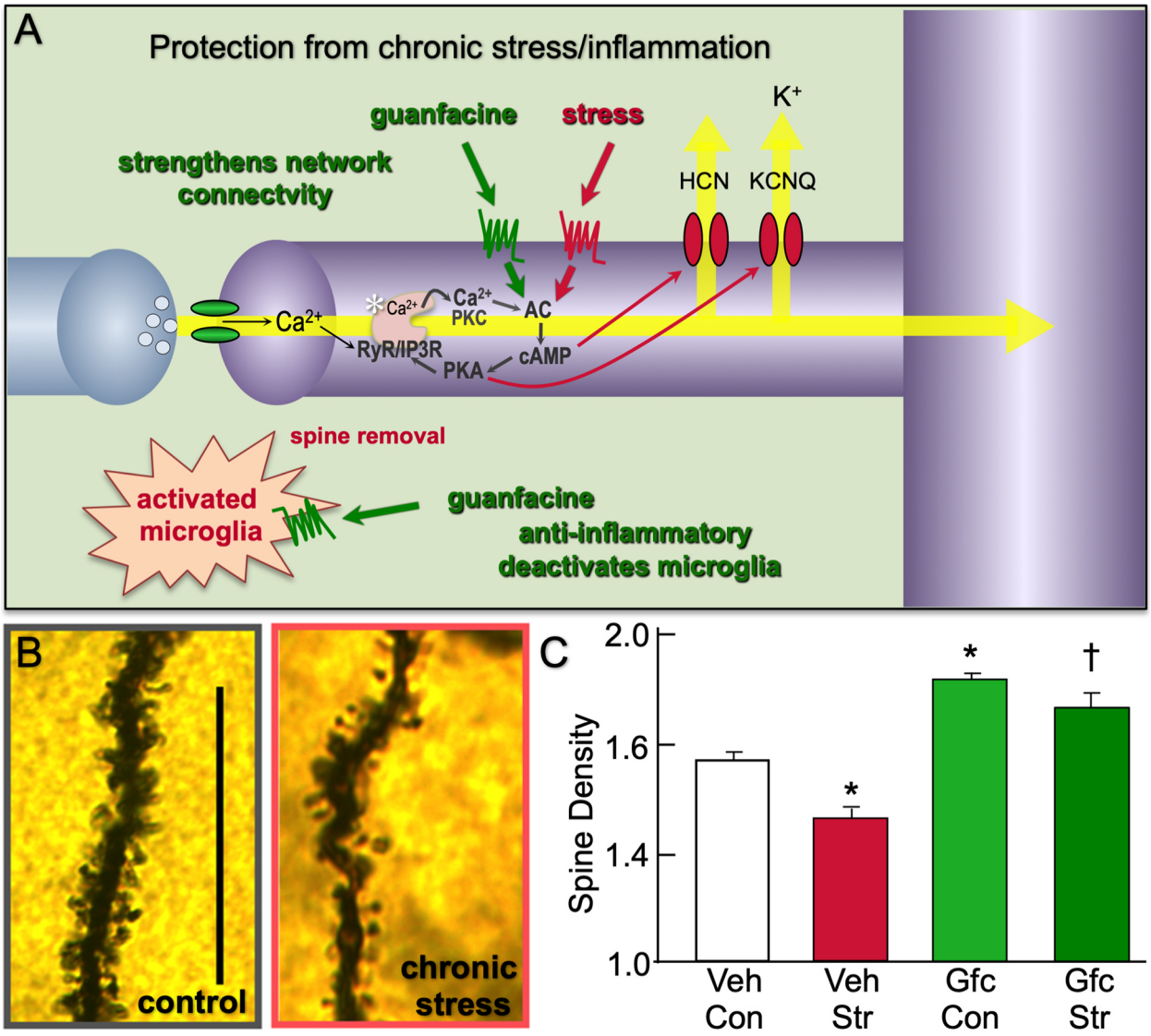

Fig. 6. Guanfacine counteracts the effects of chronic stress in PFC. (A) A schematic illustration showing how stress increases, while guanfacine inhibits, the feed-forward Ca^{2+}-cAMP-PKA-K^{+} actions that weaken PFC network connectivity, neuronal firing and function. α2A-AR are also expressed on activated microglia, where α2A-AR stimulation deactivates microglia and thus has anti-inflammatory actions. (B) Example of a layer II/III mPFC pyramidal cell distal dendrite from a control vs. chronic stressed rat, showing the reduced spine density in the stressed PFC. Scale bar indicates 25 μm. Adapted from (Hains et al., 2009) with permission. (C) Chronic stress (Str) exposure reduces PFC spine density in vehicle-treated rats, but not in those receiving daily guanfacine treatment. Con = control; Str = chronic stress; Veh = vehicle; Gfc = guanfacine. Adapted from (Hains et al., 2015) with permission.

Contant, 1992; O'Neill, Fitten, Siembieda, Ortiz, & Halgren, 2000)), a key function of the dlPFC (Suzuki & Gottlieb, 2013). A SPECT imaging study in monkeys showed that improved working memory performance was accompanied by greater activation of the dlPFC (Avery, Franowicz, Studholme, van Dyck, & Arnsten, 2000). Guanfacine also improves the ability of a young monkey to wait for a larger reward (Kim, Bobeica, Gamo, Arnsten, & Lee, 2012), a measure of impulse control, which in children is often assessed using the "marshmallow test". As described above, these abilities depend on the right inferior PFC in humans. Guanfacine also improves functions dependent on the ventral PFC. Systemic guanfacine administration in aged monkeys enhances performance of an object reversal task that requires flexible responding to reward (Steere & Arnsten, 1995), a task that Elizabeth Murray's lab at NIMH has shown depends on more ventral PFC circuits (Rudebeck, Saunders, Lundgren, & Murray, 2017). Either systemic or direct infusion of guanfacine into the ventral PFC improved the performance of monkeys performing a paired associates learning task (Wang, Ji, & Li, 2004; Wang, Tang, et al., 2004).

6.4. *Healthy humans*

Low, systemic guanfacine doses have been shown to improve performance of PFC cognitive tasks in young, healthy human subjects, with small but significant enhancing effects on working memory, paired associates learning and planning (Jakala, Riekkinen, et al., 1999; Jakala, Sirvio, et al., 1999). Guanfacine has also been shown to activate dlPFC in human functional imaging studies (Clerkin et al., 2009; Swartz, McDonald, Patel, & Torgersen, 2008). However, it can be challenging to improve cognition in healthy subjects (Muller et al., 2005), possibly related to ceiling effects on neuronal firing, as described above. Thus, most studies of guanfacine in human subjects have focused on patients with PFC disorders who would benefit most from its therapeutic actions.

7. Guanfacine treats PFC cognitive disorders in humans

A wide variety of disorders that involve PFC dysfunction are

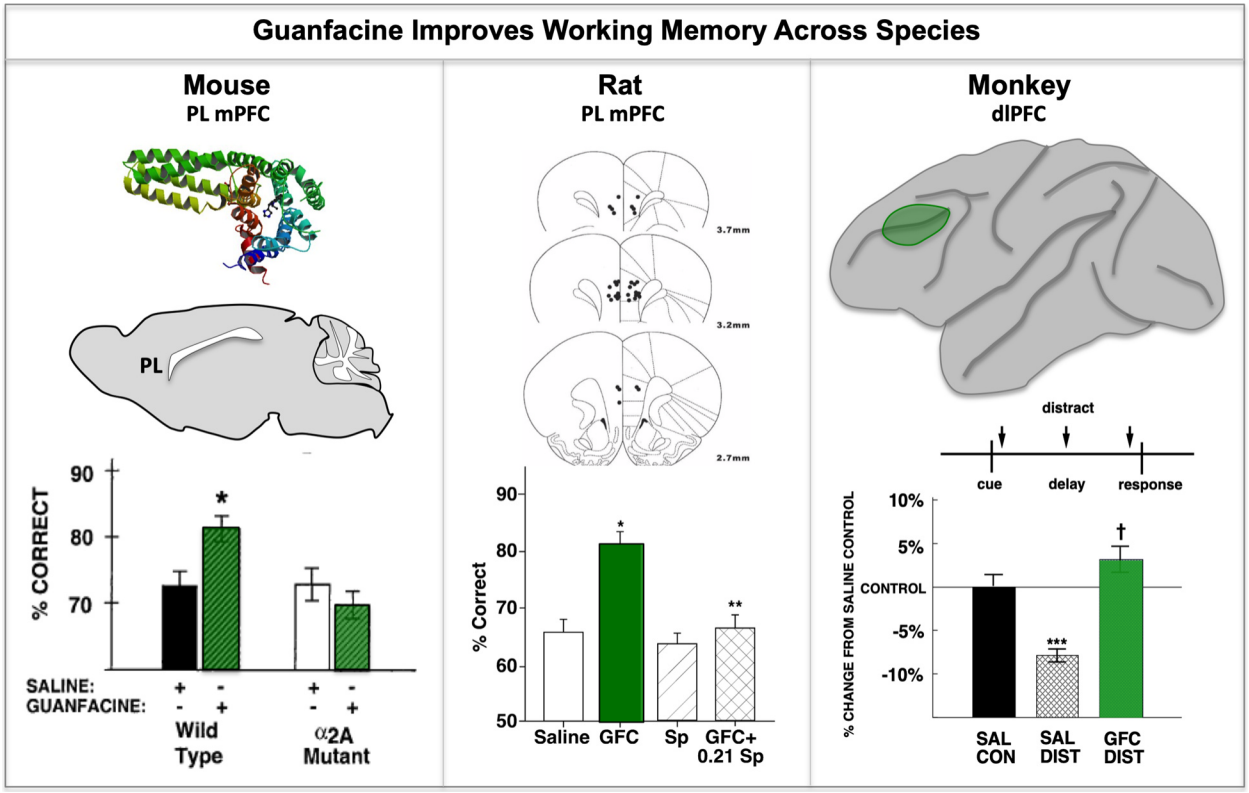

Fig. 7. Guanfacine improves working memory across species. (A) Mouse models are particularly helpful for using genetic alterations to assess molecular mechanisms. Images show the α2A-AR, and a schematic diagram of the mouse brain with the location of the prelimbic (PL) subregion of the mPFC indicated. The lower graph shows that systemic administration of guanfacine improves working memory performance in wild-type but not α2A-AR mutant mice; adapted from (Franowicz et al., 2002) with permission. (B) Infusion of guanfacine (GFC) into the aged rat PL mPFC improves working memory performance. Top- the locations of the infusion cannula tips in mPFC. Bottom- The enhancement with guanfacine is reversed by co-infusion of the cAMP-PKA agonist, Sp-cAMPS (Sp), using a low dose with no effects on its own. Adapted with permission from (Ramos et al., 2006). (C) Guanfacine improves working memory under distracting conditions in aged monkeys. Top- the location of the dlPFC subregion subserving visuospatial working memory in the macaque brain is highlit in green. Bottom- Adding distractors (DIST) during the delay period impairs performance of a working memory task if aged monkeys are pretreated with saline (SAL) but performance is protected with guanfacine (GFC) pretreatment. CON = control. Adapted with permission from (Arnsten & Contant, 1992). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

currently being treated with guanfacine, or tested in experimental studies (Fig. 8). The following is a brief review of this field.

7.1. FDA approval for the treatment of ADHD

Guanfacine is currently approved for treating ADHD in the USA, Canada, Europe and iJapan, the latter in adults (Iwanami, Saito, Fujiwara, Okutsu, & Ichikawa, 2020) as well as children. Historically, the α2-AR agonist, clonidine, was tested in ADHD, where it was thought that the powerful sedative effects of this nonselective agonist were helping to make patients less active (Hunt, Capper, & O'Connell, 1990). It is now known that the therapeutic effects are independent of, and indeed in spite of, these sedative side effects, with the development of guanfacine's use in ADHD based on understanding its prefrontal enhancement in monkeys (Arnsten & Contant, 1992; Arnsten, Steere, & Hunt, 1996), and the recognition that ADHD is a PFC disorder.

Research by Judy Rapoport, Chief of the Child Psychiatry Branch at NIMH, working with Philip Shaw, demonstrated that ADHD is associated with altered development of the right inferior PFC (Shaw et al., 2009). Functional imaging studies had also highlit dysfunction of this PFC region in ADHD e.g. studies by Casey (Casey et al., 1997) and by Rubia (Rubia, 2011) showed underactivity and/or reduced functional connectivity of the right inferior PFC as children with ADHD performed tasks requiring top down control of behavior and attention.

Guanfacine has been shown to relieve cardinal symptoms of ADHD in children and adolescents, including improved impulse control and better regulation of attention, in early, small trials (Hunt, Arnsten, & Asbell, 1995; Scahill et al., 2001), and later in multiple, large Phase III studies in the USA, Europe and Japan (Biederman et al., 2008; Hervas et al., 2014; Sallee, McGough, Wigal, Donahue, & Lyn, Biederman, Group., 2009). An early, small study of adults with ADHD showed that guanfacine could improve performance of the Stroop interference task, a task that challenges the inhibitory functions of the PFC (Taylor & Russo, 2001), and a large Phase 3 Trial showed success in adults (Iwanami et al., 2020). Guanfacine is often used as adjunct to stimulant medication, as the combination optimizes the therapeutic actions while diminishing side effects (McCracken et al., 2016). It is also used to treat patients with co-morbid ADHD and Tourette's syndrome, where stimulant medication can aggravate the appearance of tics (Scahill et al., 2001).

7.2. Use in other PFC disorders

"Off label" use of guanfacine in additional PFC disorders is widespread, but often with only limited empirical demonstrations of efficacy, as there is little corporate interest in funding large trials now that Intuniv is generically available. Interestingly, many of the disorders that are helped by guanfacine treatment have psychological or physiological stress as an etiological factor. The following is a brief review.

7.2.1. Oppositional Defiant/Conduct Disorders

These disorders of inappropriate aggression are often found in

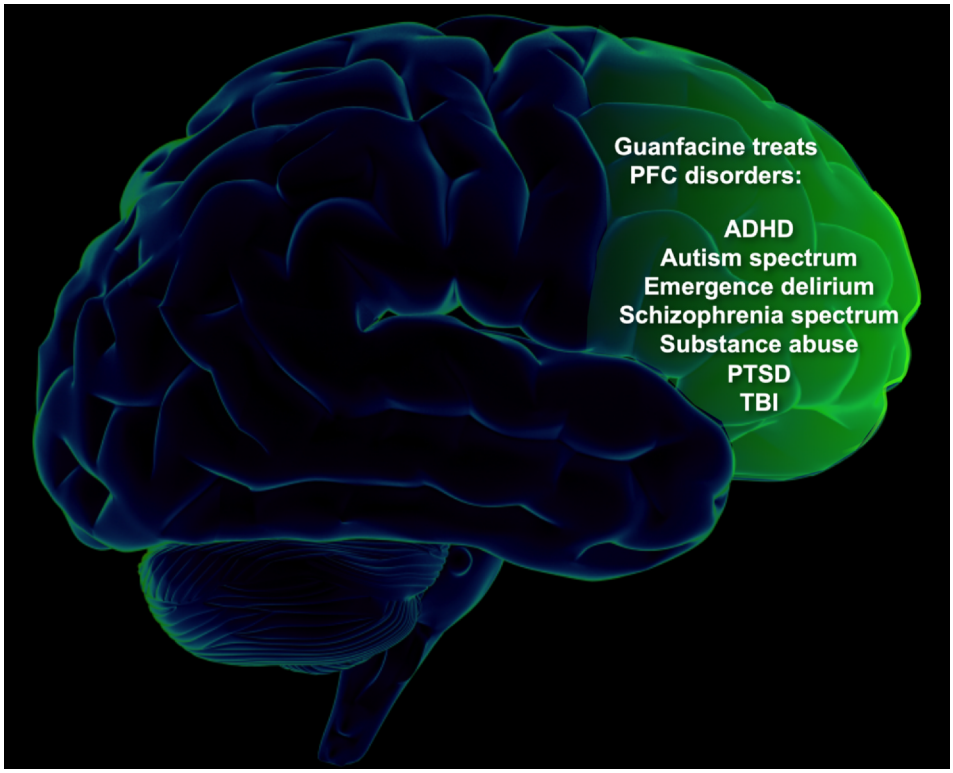

Fig. 8. Mental disorders where treatment with guanfacine is either approved for treatment, is used off-label, or is under experimental investigation.

children and adolescents who have suffered abuse. Functional imaging studies show that children with conduct disorder exhibit underactivity of more ventral PFC regions, consistent with these areas being important centers for the regulation of emotion (Rubia, 2011). Guanfacine is helpful in treating oppositional symptoms (Connor et al., 2010; Findling, McBurnett, White, & Youcha, 2014), consistent with it improving ventral PFC functions in monkeys.

7.2.2. Autism-spectrum disorders

The diagnosis of autism now represents a range of disorders of varying severity. Evidence from case reports and clinical trials indicate that guanfacine can be helpful in boosting top down control to reduce oppositional, self-injurious and repetitive behaviors in children on the autism spectrum, including children with severe (e.g. nonverbal) cognitive impairment (Coleman, Adams, Anderson, & Frye, 2019; Propper, 2018). A placebo-controlled study showed that guanfacine could be helpful for reducing oppositional and repetitive behaviors, although social behaviors are usually not improved (Politte et al., 2018).

7.2.3. Psychological trauma and abuse

Guanfacine has been especially useful in treating traumatized or abused children, who often have PFC dysfunction reflected in symptoms of impaired self-control. An open label study found that guanfacine reduced symptoms of re-experiencing the trauma, avoidance of others, and hyperarousal (Arnsten, Raskind, Taylor, & Connor, 2015; Connor, Grasso, Slivinsky, Pearson, & Banga, 2013). Guanfacine's use in adults with PTSD has been mixed. Although guanfacine has not shown efficacy in a placebo-controlled trial of veterans with longstanding PTSD (Davis et al., 2008), it may be effective in patients in earlier stages of the disorder with less severe PFC atrophy. Positive effects with guanfacine are in concert with animal studies showing that it can protect PFC connections from psychological stress exposure.

7.2.4. Traumatic brain injury, stroke, and infection

Studies in rats have shown that TBI to the posterior cortex increases PKA signaling in the PFC (Kobori et al., 2015), as well as reducing PFC spine density and impairing working memory (Zhao et al., 2018). Thus, guanfacine treatment may be helpful to normalize intracellular signaling and promote synaptic strength. Studies of humans with TBI support this view (McAllister, Flashman, Sparling, & Saykin, 2004; McAllister et al., 2011), including evidence of improved working memory and enhanced fMRI BOLD activation of PFC (McAllister et al., 2011). Guanfacine has also been shown to enhance cognition and attention in patients with impairments due to encephalitis (Singh-Curry et al., 2011), or strokes, as long as the PFC itself remains sufficiently intact to provide a therapeutic target for drug actions (Malhotra, Parton, Greenwood, & Husain, 2006).

7.2.5. Aging

Given guanfacine's benefit in aging monkeys, one would also predict predict it may be helpful in aged human subjects who, like aged monkeys, have naturally-occurring PFC cognitive deficits. A recent trial of low dose guanfacine vs. placebo in elderly humans (> 75 years) did not show significant improvement (Barcelos et al., 2018). As dendritic spines appear to lose their resilience in very old brains (Bloss et al., 2011), it may be that guanfacine treatment would need to begin at a younger age (e.g. late middle age) when the therapeutic substrate remains.

7.2.6. Substance abuse

A large number of studies indicate that guanfacine may be efficacious in treating substance abuse disorders, by strengthening PFC top-down control to resist cravings and impulsive consumption, and reducing the stress responses that often underlie drug intake.

A group of women scientists at the Yale Stress Center (Rajita Sinha, with colleagues Sherry McKee, Helen Fox and Verica Milivojevic) have shown that guanfacine can reduce stress-induced PFC dysfunction and decrease substance abuse in subjects struggling with addictions to nicotine or cocaine. McKee et al. (2015) found that guanfacine reduced stress-induced smoking in the lab, and helped people quit smoking at

home. The extended release formulation was more effective than an equivalent dose of immediate release guanacine (Verplaetse et al., 2019). Fox et al. showed that guanfacine could improve PFC functions (inhibitory control, Stroop interference) and reduce drug cravings in cocaine addicts (Fox, Sofuoglu, & Sinha, 2015; Fox et al., 2012). Guanfacine was particularly helpful in women (Milivojevic, Fox, Jayaram-Lindstrom, Hermes, & Sinha, 2017), consistent with the greater stress response in females (see above), and stress as a leading cause of substance abuse in women (McKee, Maciejewski, Falba, & Mazure, 2003).

Recent studies suggest that guanfacine may also be useful in addressing addiction to cannabis. Guanfacine treatment was shown to reduce the cognitive impairment induced by THC (Mathai et al., 2018), and new data suggest that it may be helpful in treating cannabis addiction (Dakwar et al., 2020), although there are mixed results regarding its effects on withdrawal symptoms (Haney et al., 2019; Holst et al., 2019). As cannabis use in adolescents is a risk factor for subsequent schizophrenia (Hasan et al., 2020), guanfacine treatment may also be protective in the prodrome to mental illness in high risk adolescents who are cannabis users.

7.2.7. Schizophrenia spectrum disorders

An early study suggested that guanfacine may be helpful in patients with schizophrenia who are still in early, but not later stages of the disease (Friedman et al., 2001). Studies of patients with schizophrenia are complicated by the fact that many atypical antipsychotics have complex pharmacological profiles including α2A-AR-blocking properties. However, a case report by Bardoloi (2020) suggests that guanfacine may be especially helpful in young adults with schizophrenia and a childhood history of abuse, and that it can improve insight, and thus have global benefits (Bardoloi, 2018).

Patients diagnosed with schizotypal personality disorder have cognitive deficits similar to patients with schizophrenia, including thought disorder symptoms such as ideas of reference, odd beliefs and magical thinking. However, they generally do not require antipsychotic medication, and thus can be helpful in observing the cognitive effects of potential treatments without drug interactions. Recent studies indicate that symptoms of schizotypy have a linear relationship to severe childhood trauma, and are associated with worse PFC cognitive functioning (Velikonja et al., 2019). The work of Margaret McClure has shown that guanfacine can improve working memory (McClure, Barch, Romero, Harvey, & Siever, 2007) and significantly enhance the beneficial effects of cognitive remediation (McClure et al., 2019) in schizotypal patients. The latter is particularly interesting, as a combined approach of boosting PFC physiology while engaged in cognitive training may optimize success.

7.3. Future potential uses

The research described above suggests that guanfacine may be particularly helpful during the schizophrenia prodrome, protecting PFC circuits from the psychological stressors and substance abuse (e.g. cannabis) that are such large risk factors for conversion to schizophrenia. As the descent into schizophrenic illness is accompanied by accelerated gray matter loss and signs of inflammation (Mongan, Ramesar, Föcking, Cannon, & Cotter, 2019), guanfacine's anti-inflammatory actions may be particularly helpful. The anti-inflammatory effects of guanfacine may also be useful for treating the long-term neural consequences of COVID-19, where a subset of patients report sustained mental problems (Heneka, Golenbock, Latz, Morgan, & Brown, 2020; Pinna et al., 2020).

In summary, guanfacine strengthens connectivity and enhances the firing of the newly evolved PFC circuits that underlie higher cognitive function. As these circuits expand in humans, and are subject to dysfunction in multiple mental disorders, guanfacine may be helpful in a range of cognitive disorders that benefit from improving PFC function. However, some substrate for guanfacine's beneficial actions must remain, i.e. the presence of α2A-AR -expressing PFC dendritic spines. Thus, treatment early in the course of the disorder may be essential for beneficial guanfacine actions.

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