Review Article

Caffeine, Adenosine Receptors, and Synaptic Plasticity

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Abstract. Few studies to date have looked at the effects of caffeine on synaptic plasticity, and those that did used very high concentrations of caffeine, whereas the brain concentrations attained by regular coffee consumption in humans should be in the low micromolar range, where caffeine exerts pharmacological actions mainly by antagonizing adenosine receptors. Accordingly, rats drinking caffeine (1 g/L) for 3 weeks, displayed a concentration of caffeine of circa 22 µM in the hippocampus. It is known that selective adenosine A₁ receptor antagonists facilitate, whereas selective adenosine A₂A receptor antagonists attenuate, long term potentiation (LTP) in the hippocampus. Although caffeine is a non-selective antagonist of adenosine receptors, it attenuates frequency-induced LTP in hippocampal slices in a manner similar to selective adenosine A₂A receptor antagonists. These effects of low micromolar concentration of caffeine (30 µM) are maintained in aged animals, which is important when a possible beneficial effect for caffeine in age-related cognitive decline is proposed. Future studies will still be required to confirm and detail the involvement of A₁ and A₂A receptors in the effects of caffeine on hippocampal synaptic plasticity, using both pharmacological and genetic approaches.

Keywords: A₁ receptors, A₂A receptors, adenosine, aging, caffeine, long-term potentiation, hippocampus, synaptic plasticity

INTRODUCTION

The cognitive enhancing properties of caffeine have long been recognized, and are experienced daily by regular coffee drinkers. Although it has been more difficult to demonstrate these cognitive enhancing effects in a formal experimental setting, caffeine improves mood and enhances psychomotor and cognitive performance in healthy volunteers, particularly on tasks measuring typing speed, simple reaction time, sustained attention, memory, and logical reasoning, as well as simulated driving [1]. Since it is generally accepted that the neurophysiological basis for learning and memory involves long-term modifications in the efficiency of synapses integrated in networks caused by specific patterns of neuronal activity [2], it would appear obvious to test whether caffeine could facilitate long-term potentiation (LTP) and related phenomena of synaptic plasticity in brain areas important for learning and memory.

WHAT IS PRESENTLY KNOWN ABOUT THE EFFECTS OF CAFFEINE ON SYNAPTIC PLASTICITY?

Rather surprisingly, few studies looked at the effects of caffeine on synaptic plasticity. In fact, re-
experiments were 500 synapses; the concentrations of caffeine tested in these tetanic potentiation, but did not affect LTP, at the same increase basal synaptic transmission and inhibit post-tetanic potentiation, but did not affect LTP, at the same synapses; the concentrations of caffeine tested in these experiments were 500 µM and 10 mM [4]. Thus, the concentrations of caffeine used in these two studies were several orders of magnitude higher than the concentration of caffeine attained in the plasma in humans by ingestion of moderate amounts of coffee, estimated to be 5–70 µM (see [5–8]). The only pharmacological action known for caffeine at the low micromolar range is the antagonism of adenosine receptors, namely adenosine A₁ and A₂A receptors [9,10], and possibly adenosine A₃ receptors [11]. Other effects, like inhibition of phosphodiesterases, intracellular calcium release and blockade of GABA_A receptors, require higher concentrations of caffeine [10,12], although future studies still need to be carried out to either exclude or define the role of these putative targets in the central effects of caffeine. In any case, the estimation of the effects of acutely applied caffeine on LTP will first require establishing the plasma and brain concentrations of caffeine.

WHAT CONCENTRATIONS SHOULD BE TESTED TO REFLECT THE CAFFEINE LEVELS IN THE BRAIN?

We have previously found that the administration of caffeine through the drinking water at a concentration of 1 g/L affords neuroprotection and prevents memory deficits in rodents upon different noxious brain insults mimicking conditions such as diabetes, stress, early life convulsions, or Alzheimer’s disease [13–15]. When allowed to have free access to caffeine in their drinking water (1 g/L) for a period of 4 weeks, rats consumed an average amount of 73 ± 3 mg caffeine/kg/day (estimation based on the average daily consumption of caffeinated water of the 6 animals during the last 2 weeks of the treatment). Upon HPLC evaluation (see e.g. [14]), these rats displayed a plasma concentration of caffeine of 32 ± 4 µM (n = 6), which is equivalent to the plasma concentration of caffeine found in humans consuming circa 6 cups of coffee daily [5,7,8]. In the same rats, the concentration of caffeine in the cerebrospinal fluid was lower than in the plasma, reaching a value of 23 ± 3 µM (n = 6). Finally, 0.409 ± 0.011 µg of caffeine were recovered in the hippocampal extracts (n = 6); this corresponds to an estimated concentration of 22 µM of caffeine, assuming a total hippocampal volume of 95 µL [16,17]. Since the brain concentration of caffeine is within 80% of its plasma concentration, we would recommend a caffeine concentration of about 30 µM to probe the effects of caffeine on synaptic plasticity in hippocampal slices.

EFFECTS OF ADENOSINE A₁ RECEPTOR ANTAGONISTS AND ADENOSINE A₂A RECEPTOR ANTAGONISTS ON SYNAPTIC PLASTICITY

Since, as mentioned above, caffeine is a non-selective adenosine A₁ and A₂A receptor antagonist, the putative effects of relevant concentrations of caffeine on synaptic plasticity could to a certain extent be inferred from the known effects of selective adenosine A₁ receptor antagonists and selective adenosine A₂A receptor antagonists on synaptic plasticity. The modulation exerted by adenosine receptors on synaptic plasticity was previously reviewed [18], but a summary of the key finding will be presented.

 Usually compounds that are agonists of adenosine A₁ receptors, like adenosine itself, attenuate LTP, whereas selective antagonists of A₁ receptors, like 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), facilitate LTP [18]. This facilitatory effect of A₁ receptor antagonists is more evident when using relatively weak LTP induction protocols, namely theta burst stimulation, and may be more difficult to detect when using strong stimulation protocols, namely high frequency stimulation, closer to LTP saturation conditions. For example, a theta-burst stimulation protocol (only 3 trains of 3 stimuli each) could not elicit LTP under control conditions in one pathway at Schaffer fibers/CA1 pyramid synapses in vitro, but was able to induce robust LTP either in
the same pathway, or in the other convergent pathway, after adding DPCPX (50 nM) to the superfusion medium [19]. The same selective adenosine receptor antagonist could restore the LTP decay observed in Schaffer fibers/CA1 basal dendrite synapses in middle-aged animals [20]. This contrasts with our recent observations that DPCPX has discrete effects on LTP amplitude in middle aged rats and is virtually devoid of effects on LTP amplitude in hippocampal slices from aged rats [21].

The effects of compounds that act upon adenosine A$_{2A}$ receptors on phenomena of synaptic plasticity have been initially somewhat inconsistent, possibly because of the moderate selectivity of the antagonists available at that time [18]. The A$_{2A}$ receptor agonist, CGS 21680, facilitated LTP at Schaffer fibers/CA1 pyramid synapses in hippocampal slices [22,23]. When tested at these same synapses, the highly selective A$_{2A}$ receptor antagonist, SCH 58261 (50 nM), attenuated LTP in vitro [21] and in vivo [24], an effect which was more prominent in aged animals [21]. The same A$_{2A}$ receptor antagonist also blocked LTP of N-methyl-D-aspartate (NMDA) mediated responses at mossy fibers/CA3 pyramid synapses in hippocampal slices [22,23]. When these more recent data, taken together, suggest an important role for A$_{2A}$ receptors in controlling LTP at distinct hippocampal synapses.

Regarding adenosine A$_3$ receptors, their level of expression in the brain is low [26], albeit they are located in hippocampal nerve terminals [27]. Nevertheless, one study showed that an adenosine A$_3$ receptor agonist, CI-IBMECA (100 nM), could increase LTP at Schaffer fibers/CA1 pyramid synapses in vitro, an effect prevented by a selective A$_3$ receptor antagonist, MRS 1191 (10 µM). The antagonist was itself devoid of effect, suggesting that endogenous adenosine does not tonically modulate LTP in the hippocampus by activating A$_3$ receptors [28].

In conclusion, selective adenosine A$_1$ receptor antagonists facilitate, whereas selective adenosine A$_{2A}$ receptor antagonists attenuate, LTP in the hippocampus. Pharmacological studies have shown that caffeine is a low affinity antagonist of both A$_1$ and A$_{2A}$ receptors, with slightly higher affinity for A$_{2A}$ receptors in native brain preparations (e.g. [29]). Based on these pharmacological properties of caffeine, it would be difficult to predict what could be its action on LTP at concentrations comparable to those attained by regular consumption in humans.

Preliminary evidence from our laboratories suggests that caffeine attenuates frequency-induced LTP in hippocampal slices in a manner similar to selective adenosine A$_{2A}$ receptor antagonists. Electrophysiological recordings of field excitatory postsynaptic potentials (fEPSP) were obtained from hippocampal slices taken from male Wistar rats as previously described [19]. Two separate sets of the Schaffer fibers/CA1 pyramid synapses (S1 and S2) were alternately stimulated at a basal frequency of 0.05 Hz. LTP was induced by high-frequency stimulation pattern (HFS, 1 train of 100 Hz, 100 stimuli) in one pathway (control) under control conditions, and afterwards in the other pathway (test) in the presence of caffeine (30 µM). Caffeine was added at least 45 minutes before applying the HFS train to the test pathway and was present throughout the rest of the experiment. As depicted in Fig. 1A, when a HFS train was delivered to hippocampal slices taken from young adult rats (10–15 weeks), there was a sustained increase of synaptic efficiency, i.e., there was a LTP with a magnitude of 97.0 ± 3.8% over baseline (LTP quantified as the % increase in the average value of the fEPSP slope taken from 50 to 60 min after HFS in relation to the average value of the fEPSP slope during the 10 min that preceded HFS; n = 5; Fig. 1A). Caffeine (30 µM) decreased the magnitude of HFS-induced LTP to 53.4 ± 7.3% over baseline (n = 5, P < 0.05; paired two-tailed Student’s t test; comparing the LTP values in the absence and in the presence of caffeine; Fig. 1A).

Although the analogy of the inhibitory effects of caffeine on high frequency-induced LTP with the known inhibitory effects of selective adenosine A$_{2A}$ receptor antagonists is striking, it should be experimentally confirmed in the future. The prediction would be that the inhibitory effects of caffeine on LTP would be occluded when a selective adenosine A$_{2A}$ receptor antagonist is applied throughout the entire experiment.

**ROLE OF ADENOSINE A$_3$ AND A$_{2A}$ RECEPTORS IN YOUNG AND AGED ANIMALS**

Similar experiments were performed in slices derived from aged rats (70–80 weeks old). HFS stimulation also triggered LTP, which reached a magnitude of 99.8 ± 7.3% over baseline in control conditions (n = 4; Fig. 1B). As occurred in young adult rats, caffeine (30 µM) decreased the magnitude of HFS-induced LTP to 36.3 ± 8.2% of baseline (n = 4, P < 0.05; paired two-tailed Student’s t test; Fig. 1B). These preliminary results thus suggest that the inhibitory effect of caffeine on the control of LTP magnitude is maintained both in young adult and in aged animals. Again it is hy-
Caffeine inhibits long-term potentiation (LTP). Caffeine (30 µM, filled symbols) decreased the magnitude of high frequency stimulation (HFS)-induced long-term potentiation (LTP) of the field excitatory postsynaptic potentials (fEPSP) in the Schaffer pathway in hippocampal slices from both young adult (A) and aged rats (B). Data are mean ± 1SEM of 4–5 experiments carried out in slices from different rats. *P < 0.05, LTP significantly different between control (absence of added drugs; open symbols) and the presence of 30 µM caffeine (filled symbols), Student’s paired t test.

The authors hypothesised that A$_{2A}$ receptors may be the main target of caffeine to control of LTP in aged rats since there is an age-associated increase of A$_{2A}$ receptor density and function, associated with the decreased A$_1$ receptor density and function [21,30–33]. Thus, the effects of caffeine on LTP are preserved upon aging, a conclusion of main importance for the interpretation of the beneficial effects of caffeine in neurodegenerative conditions that are prevalent in the elderly.

**DIFFERENTIAL ROLE OF ADENOSINE A$_1$ AND A$_{2A}$ RECEPTORS UNDER CONDITIONS OF BASAL FREQUENCY STIMULATION AND HIGH FREQUENCY STIMULATION**

Under conditions of high frequency-induced synaptic plasticity, endogenous extracellular adenosine mainly activates facilitatory A$_{2A}$ receptors; in contrast, under conditions of basal frequency synaptic transmission, endogenous extracellular adenosine predominantly activates inhibitory A$_1$ receptors (reviewed in [34]). Since the effects of caffeine could be largely directed by the physiological conditions that determine whether the predominant signaling is dominated by A$_1$ receptors or by A$_{2A}$ receptors (both of which can be antagonized nearly equi-efficiently by caffeine), understanding the different (and complementary) roles of A$_1$ and A$_{2A}$ receptors in the control of basal frequency synaptic transmission and synaptic plasticity is required.

A series of different approaches have allowed devising a scenario supporting a differential role for A$_1$ and A$_{2A}$ receptors in the control of basal frequency synaptic transmission and plasticity, which is essentially based on the different sources of adenosine responsible for the activation of both receptors together with a crosstalk between A$_1$ and A$_{2A}$ receptors (reviewed in [34]). Thus, initial studies combining biochemical, neurochemical and electrophysiological studies have supported the idea that the activation of A$_{2A}$ receptors seems to require a particular source of adenosine, which is formed from the action of synaptically-located ecto-nucleotidases [35] upon extracellular catabolism of synaptically-released ATP [25,36]. Interestingly, the release of ATP from activated nerve terminals occurs in small amounts at low frequencies of stimulation but occurs in disproportionally larger amounts upon high frequency stimulation of nerve terminals [37–39]. This provides a neurochemical basis for the selective engagement of A$_{2A}$ receptors at high rather than low frequencies of nerve stimulation [34], since only during the former, but not the latter, conditions is there a localised synaptic formation of ATP-derived adenosine able to activate the A$_{2A}$ receptors that are highly en-
riched in synapses in the hippocampus [40]. In contrast, A1 receptors are effectively activated by extracellular adenosine, which is either released as such or formed from ATP released from astrocytes [41] in a manner dependent on global nerve activity [42]. Thus, under basal frequency stimulation, there is an increase of extracellular adenosine levels [42] and an on-going inhibitory tonic provided by activation of inhibitory A1 receptors [43]. This A1 receptor system is rather effective and able to completely block glutamatergic synaptic transmission in the hippocampus if maximally activated (see e.g. [44]). This poses a problem to understand the possibility of increasing the efficiency of hippocampal synapses, for instance upon high frequency stimulation (LTP). In other words, increased frequency of stimulation enhances the production of extracellular adenosine [42], which would be expected to fully block synaptic transmission through A1 receptor activation. Therefore, the expression of enhanced synaptic transmission upon high frequency stimulation should require switching off A1 receptors. Interestingly, we have found that the activation of A2A receptors is able to decrease functioning of A1 receptors through alternative (or combined) mechanisms involving a protein kinase C-mediated desensitization of A1 receptors [45, 46] and a heteromerization of A1 and A2A receptors whereby the activation of A2A receptors switches off A1 receptors [29]. Merging these two lines of evidences presented above, one ends up with a likely scenario able to explain the predominant role of A1 receptors under basal frequency stimulation and a predominant role of A2A receptors in synapses undergoing plasticity changes upon high frequency stimulation (reviewed in [34]).

Adenosine A1 receptors are constitutively activated in all glutamatergic synapses and act as a general threshold barrier that tonically restraints synaptic transmission and, to a lower extent, LTP (reviewed in [34]). In particular at synapses undergoing plastic changes, there is a large ATP release and the resulting locally increased activation of A1 receptors in all synapses (except the activated synapses, where A1 receptors are switched off) contributes to define salience in complex neuronal circuits where only one group of synapses is activated to encode relevant information (reviewed in [34]).

Following the previous discussion, we might expect that caffeine, beyond antagonizing adenosine A2A receptors to modulate high frequency-induced synaptic plasticity, would also antagonize adenosine A1 receptors to control basal frequency synaptic transmission. Although this has not yet been confirmed experimentally, the available evidence is compatible with this hypothesis. Thus, caffeine, at low micromolar concentrations, is known to increase basal frequency synaptic transmission in the Schaffer fibers likely through antagonism of A1 receptors [50]. Also in experiments from our laboratories, when applied acutely to hippocampal slices obtained from young adult rats (10–15 weeks), stimulated at a basal frequency of 0.05 Hz, caffeine (30 µM) caused a facilitation of synaptic transmission that reached an amplitude of 34.8 ± 5.9% of baseline (facilitation quantified as the % increase in the average value of the fEPSP slope taken from 20 to 35 min after beginning application of caffeine in relation to the average value of the fEPSP slope during the 10 min that preceded application of caffeine; n = 5, P < 0.05; paired two-tailed Student’s t test; Fig. 2A). In a similar way, in slices derived from aged animals (70–80 weeks old), caffeine (30 µM) also enhanced basal frequency synaptic transmission, a facilitation that reached an amplitude of 55.7 ± 8.0% of baseline (n = 5; P <0.05; paired two-tailed Student’s t test; Fig. 2B). This facilitatory effect of caffeine on basal synaptic transmission is qualitatively similar to that obtained when applying a selective A1 receptor antagonist or upon removal of endogenous extracellular adenosine (using adenosine deaminase) in either young adult or aged rats [31].

POSSIBLE RELEVANCE OF THE EFFECTS OF CAFFEINE ON LTP FOR MEMORY PERFORMANCE

Apart from its intrinsic scientific interest, the determination of the effects of caffeine on synaptic transmission and plasticity are largely driven by the striking ability of chronic caffeine consumption to prevent memory dysfunction in several animal models of disease [51,52]. This also seems to occur in humans, where it is observed that caffeine consumption...
Fig. 2. Caffeine facilitates basal synaptic transmission. A) Caffeine (30 µM) enhanced the amplitude of the field excitatory postsynaptic potentials (fEPSP) in the Schaffer pathway upon basal stimulation (0.05 Hz) of hippocampal slices both from young adult (A) and from aged rats (B) in a manner similar to that caused by administration of selective A$_1$ receptor antagonists (data not shown). Data are mean ± 1SEM of 5 experiments carried out in slices from different rats. *P < 0.05, comparing the mean fEPSP slopes at different time points with the average value of the fEPSP slope during the 10 min that preceded application of caffeine, n = 5. Student’s two-tailed paired t test, no correction made for multiple comparisons.

may prevent age-related cognitive impairment [53] (but see [54]) and may be inversely associated with the incidence of Alzheimer’s disease [55,56]. Interestingly, animal studies have largely concluded that the benefits afforded by chronic caffeine consumption in terms of attenuating memory impairment upon several noxious stimuli [51,52] are mainly mimicked by selective antagonists of A$_{2A}$ rather than A$_1$ receptors. Given that LTP has been conceived as a possible neurophysiological correlate of learning and memory (see [2,57]), the present observation that the effect of caffeine on LTP is qualitatively similar to the effects of selective A$_{2A}$ receptor antagonists bolsters the hypothesis that this effect of caffeine on LTP may be related to the ability of caffeine to attenuate the impairment of memory performance. This parallel between the memory-restoring properties of caffeine (possibly through A$_{2A}$ receptor antagonism) and the presently observed inhibition of LTP by caffeine (also possibly through A$_{2A}$ receptor antagonism) makes it important to discuss a general incorrect assumption about a parallel relationship between the magnitude of LTP and memory performance. This widespread idea probably arose from the pioneering observations that manipulations blocking LTP also severely reduced performance in learning and memory-related tasks (reviewed in [2]). However, this sustains, at best, that LTP is required for memory processing but it should not be taken as evidence that LTP and memory are linearly related. In fact, it has previously been elegantly discussed that correct processing of memory-related information should be dependent on defining the salience of information to be encoded, a process that requires not only potentiation of synaptic strength but also depression and depotentiation (e.g. [2,58,59]), both homosynaptic and heterosynaptic (e.g. [60,61]). For that reason, there are several previous manipulations of different proteins that resulted in an enhanced LTP coupled to decreased performance in memory-related tasks (e.g. [62–66]). Thus, it is understandable that caffeine (possibly through A$_{2A}$ receptor antagonism) causes a decrease of LTP magnitude while restoring memory dysfunction, if one considers that caffeine and A$_{2A}$ receptor antagonists act as normalizers rather than facilitators of synaptic plasticity and memory (discussed in [34]; see also Cunha and Agostinho, this issue).

This discussion should not exclude the possibility that the effects of caffeine on memory performance might target brain structures apart from the hippocampus and involve the control of processes that indirectly regulate memory performance. For instance, caffeine improves sustained attention [1], and attention effectively modulates the encoding and long-term retrieval of memories (e.g. [67,68]). However, since it has been shown that caffeine can have additional effects on memory performance apart from its effects on
It should be noted that, so far, it was observed that caffeine attenuates frequency-induced LTP in the Schaffer pathway, but it would be interesting to test if similar effects of caffeine are also present in different excitatory synapses, particularly at the mossy fibers/CA3 pyramid synapses, where a selective adenosine A$_{2A}$ receptor antagonist could block LTP of NMDA receptor-mediated responses [25].

Although we report that caffeine controlled LTP amplitude when induced by high frequency stimulation, there are several patterns of frequency-induced synaptic plasticity (e.g. theta-burst stimulation) where possible effects of caffeine remain to be determined. In this respect, it should be emphasized that the activation of adenosine A$_{2A}$ receptors is not expected to be a mandatory process to implement LTP, unlike the recruitment of NMDA receptors. Instead, A$_{2A}$ receptors are expected to assist (or facilitate) the implementation of LTP. And it still remains to be explored under which conditions of plasticity A$_{2A}$ receptors might play determinant or passive roles. It must also be taken into account that caffeine is expected to cause a partial blockade of A$_{2A}$ receptors, in contrast to selective A$_{2A}$ receptor antagonists, which were used at a supramaximal (but selective) concentration to nearly fully block A$_{2A}$ receptors. For instance, the engagement of A$_{2A}$ receptors at the neuromuscular junction was found to be possible only with intermittent burst of high frequency stimulation rather than with a single continuous train of high frequency stimulation [71], which might be related to a different pattern of ATP release with distinct types of synaptic plasticity induction protocols that may determine the recruitment of A$_{2A}$ receptors.

In the in vitro experiments on synaptic plasticity described, caffeine is usually applied in the bath superfusing the slices. It would be important to know how a prolonged and intermittent ingestion of caffeine in the experimental animals, mimicking the caffeine consumption pattern in humans, would affect synaptic plasticity elicited in slices taken from these animals. In vivo experiments in animals with chronically implanted electrodes would be particularly relevant for that purpose.

Another interesting point is that we have determined the effects of caffeine in male rats but it is not known what might be the effect of caffeine in female rats. This question is particularly important since there are numerous reports of sex differences in the ability of caffeine to afford neuroprotection. For instance, there is an inverse association between caffeine intake and the incidence of Parkinson’s disease, which is more evident in men than in women [72], possibly due to the interfering effect of estrogen replacement therapies [73], as also suggested in animal studies [74]. In contrast, the age-related cognitive decline was attenuated by caffeine consumption in women in one study [53], whereas another study reported an inverse association between caffeine intake and the risk of dementia that was stronger in men [56]. Future work should explore whether there are differences between the effects of caffeine both on learning and memory performance and on synaptic plasticity between males and females, to help clarify this sex difference issue.

Finally, it would be of interest to test if caffeine selectively modulates long term depression and depotentiation as well as other types of shorter or longer plasticity phenomena, and to explore the mechanisms linking the control by caffeine of synaptic plasticity and its ability to modulate learning and memory performance.

CONCLUSIONS

Concentrations of caffeine above 50 $\mu$M, used in previous studies describing the effects of caffeine on LTP, are probably not representative of the brain concentrations of caffeine obtained by ingestion of moderate amounts of coffee and caffeine-containing beverages in humans. The present study provides the first description that relevant concentrations of caffeine inhibit the magnitude of LTP triggered by high-frequency stimulation (possibly via A$_{2A}$ receptors), in contrast to the excitatory effects of caffeine on basal synaptic transmission (possibly via A$_1$ receptor antagonism). These effects of caffeine appear to be maintained in aged animals, possibly as a result of balanced change of the density and efficiency of A$_1$ and A$_{2A}$ receptors [30–33] and of the sources of endogenous extracellular adenosine [75]. This observation is of particular importance when considering the possible beneficial effect for caffeine in age-related cognitive decline. Future studies will still be required to confirm and detail the involve-
ment of $A_1$ and $A_{2A}$ receptors in the effects of caffeine on hippocampal synaptic plasticity, using both pharmacological and genetic approaches.

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