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The complexity of small circuits: the stomatogastric nervous system

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Abstract

The crustacean stomatogastric nervous system is a long-standing test bed for studies of circuit dynamics and neuromodulation. We give a brief update on the most recent work on this system, with an emphasis on the broader implications for understanding neural circuits. In particular, we focus on new findings underlining that different levels of dynamics taking place at different time scales all interact in multiple ways. Dynamics due to synaptic and intrinsic neuronal properties, neuromodulation, and long-term gene expression-dependent regulation are not independent, but influence each other. Extensive research on the stomatogastric system shows that these dynamic interactions convey robustness to circuit operation, while facilitating the flexibility of producing multiple circuit outputs.

Introduction

Studying neural circuits comes with a number of technical and conceptual challenges [1]. Any given circuit is not equally amenable to all technical approaches, which makes bridging levels of analysis difficult. In addition, numerical complexity, poorly defined cell types, and incomplete connectivity maps often make inferences from cellular to circuit function tentative at best. Furthermore, establishing functional boundaries for circuits embedded in larger brain areas can be difficult. Some of these problems are less severe in invertebrate preparations, which for this reason have been useful in unraveling evolutionarily conserved principles of circuit operation.

The stomatogastric nervous system (STNS) stands out for its utility in studying how neuronal and synaptic properties give rise to circuit activity and are shaped by neuromodulation and other regulatory processes [2]. The pattern-generating circuits of the STNS play an important role in feeding in all arthropods. However, the insect STNS has been studied mainly from a developmental and anatomical perspective [3], and although

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some of the activity patterns and neuromodulators involved in regulating feeding have been studied [4–6], the neural circuits that underlie these activities are as yet unidentified. Consequently, we will focus on the crustacean STNS.

In lobsters and crabs, the STNS is a conveniently anatomically separated system of a few ganglia that controls rhythmic activity of the foregut and can easily be studied *in vitro*. The stomatogastric ganglion (STG) contains only ~30 neurons, comprising two overlapping central pattern generating circuits that produce the slow gastric mill rhythm and the faster pyloric rhythm (Fig. 1). The neurons are large and easily identifiable, and their connectivity has long been established. Considering the ongoing efforts in connectomics in many systems, it is humbling that such connectivity diagrams (Fig. 1B) provide little explanation of circuit activity and dynamics. This is due to the nonlinear dynamics of membranes and synapses, neuromodulation, and long-term regulation, all of which can influence circuit activity over multiple time scales [7]. Here we review recent work on several aspects of these different time scales of circuit dynamics in the STG, and in particular how processes at different time scales interact.

Dynamics arising from intrinsic and synaptic properties

The pyloric rhythm is based on intrinsic oscillatory properties of a pacemaker kernel, and follower neurons burst in rebound from inhibition by the pacemaker [8] (Fig. 1B&C). The gastric mill rhythm arises from synaptic connectivity of non-oscillatory neurons [9] (Fig. 1B&D). The intrinsic neuronal and synaptic properties are well described in the STG, but it is not necessarily obvious how these components function within the context of circuit activity. Dynamics arising from the interactions of synaptic inputs and postsynaptic properties have recently been studied experimentally and theoretically in the context of how inhibitory feedback from follower neurons affects the pyloric pacemaker oscillation. At its usual timing with respect to the phase of oscillation, feedback inhibition has surprisingly little effect on the mean period of the rhythm, but reduces cycle-to-cycle variability and therefore stabilizes oscillations [10–12]. Similar stabilizing influences of synaptic input on irregularly firing neurons have also been theoretically demonstrated for network-based oscillations [13]. The effect of timing of synaptic input with respect to the phase of ongoing activity also allows analyzing the contributions of specific ionic conductances [14]. Another window into how neuronal and synaptic properties shape circuit activity is provided by the observation that pyloric neurons and synapses have preferred frequencies, i.e. show best responses at specific input frequencies. The distinct frequency preferences of different network components are correlated with the period of the rhythm and potentially the phasing of neurons, and are altered when neuromodulators change circuit activity [15*].

Neuromodulation

The STNS is perhaps best known for its role in uncovering principles of neuromodulation. Metabotropic actions of neuromodulators are at the root of the ability of circuits to produce different activity patterns [16–18]. The pyloric rhythm is continuously active and its stereotypical triphasic activity (Fig. 1C) can be configured by neuromodulators *in vitro* into different temporal patterns. The gastric mill rhythm is often not spontaneously active, but

can be activated by modulatory projection neurons to generate distinct patterns (Fig. 1D). *In vivo* studies show that pyloric activity is indeed changed after feeding, and that distinct gastric mill rhythms exist in the intact animal [19*,20]. This flexibility stems from the fact that the STG is affected by a large number of neuromodulators, including classic neurotransmitters, biogenic amines, and many neuropeptides, which are either released from descending projection neurons, or present in the hemolymph. Even considering substantial flexibility, the sheer number of neuropeptides (>100) is puzzling. However, some isoforms of the same family may activate promiscuous receptors and not have distinct actions[21,22]. Great strides have been made identifying neuropeptides with mass spectrometry, and it is now possible to quantitatively map them to specific tissue regions in individual animals [23*, 24], or detect abundance changes in hemolymph after feeding [25].

The circuit-wide actions of biogenic amines and a few neuropeptides have been studied thoroughly, and show fairly distinct organizing principles (Fig. 2). Amines have divergent cellular and synaptic actions, i.e. modulator and cell type-specific effects on different subsets of multiple ion channel types across all neurons [16] (Fig. 2A&B). Different amines may all affect every single neuron and synapse, but the sum of effects on their multiple subcellular targets is different. Such divergent actions extend even to differential effects on synaptic dynamics [26]. Neuropeptides, on the other hand, have mostly convergent actions on a limited set of intracellular targets. In particular, they all activate the same modulator-activated inward current (I_{MI}) [27] (Fig. 2C) which shows unusual voltage-dependence in that it is regulated by both intra- and extracellular calcium [28]. This single current represents a powerful way to activate the pyloric circuit [29], and recent experimental and theoretical work shows that just the negative slope conductance of its IV curve is sufficient to elicit oscillatory activity [30,31]. The specificity of effects of different neuropeptides stems from the fact that each activates I_{MI} in a different subset of neurons [32] (Fig. 2D). In addition, specific effects can arise from differences in the temporal structure of release [33], and from cell type-specific differences in receptor expression levels and associated differences in the magnitude of I_{MI} responses [34**]. On the flip side, different modulatory inputs can also result in very similar circuit activity, as the same gastric mill rhythm can arise from distinct rhythm-generating mechanisms configured by different neuropeptides [35**]. Many neuropeptides exist as co-transmitters in projection neurons, which in effect modify their actions. The spatial pattern of release may also matter, as co-transmitters can be released differentially into different target areas [36,37].

Neuromodulation does not just affect neural circuits, but also their inputs and outputs. Input from modulatory projection neurons can be shaped by feedback from the CPG, which in turn alters CPG activity [38*]. Such bidirectional interactions themselves are modifiable, because the feedback synapses from CPG to projections neurons can also be modulated [39]. At the output level, neuromodulation does not just affect how activity is generated, but potentially also how it is propagated, as axonal spike conduction can be altered by modulators [40].

Variability and regulation of stable circuit activity

All STG neurons have qualitatively similar complements of ionic currents, but differences in their relative magnitude convey distinct intrinsic properties. In consequence, the contribution

of a given ionic current can differ across neurons within one circuit, or between pyloric and gastric mill neurons [41]. The characteristic intrinsic properties of each cell type, manifest in stereotyped voltage trajectories and consistent responses to input, is surprisingly not due to tightly controlled expression levels of individual ionic currents. Current densities and mRNA expression for channel genes in a cell type can be hugely variable across individuals, even when the cell type includes only a single neuron, which raises the question how cell identities and physiological phenotypes are maintained. Stable output in the face of component variability is thought to stem from homeostatic regulatory mechanisms that allow individual animals to reach one of many permissive parameter combinations, and research using the STNS has been on the forefront of exploring this issue [42].

Ionic currents do not vary independently, but cell type-specific groups of ion channel types often covary, which is thought to have compensatory function for the variability [43,44]. Such co-variation of ionic conductances can keep features of pyloric neuron activity invariant and robust to perturbations [45], and are maintained by activity-dependent feedback [46**]. These experimental findings have inspired a series of theoretical studies that show that co-variation can support maintenance of firing phase across neurons [47], and that simple homeostatic tuning rules can easily incorporate conductance correlations [48] and may in fact depend on these correlations to give rise to stable circuit activity [49**]. Regulation of synaptic strength and dynamics may also be involved in compensating for variable circuit architectures and neuronal properties [50,51], but it is not clear to which degree they may covary with other parameters.

Neuromodulation and long-term regulation of circuit properties

Given that circuit components are both variable across individuals and malleable to many neuromodulators, it is surprising that circuit activity is robust and neuromodulators cause consistent activity changes across individuals [16,52,53]. However, at least in the long term, neuromodulation does not seem to be the cause of variability, but can reduce it. When neuromodulatory inputs are removed from the STG, rhythmic activity can slow or cease. Over the course of tens of hours, circuit properties are then reconfigured. Such reconfiguration can be sufficient to recover pyloric activity [54,55], but more recent work shows that short of recovery, long-term removal of neuromodulators and subsequent changes in circuit components increases variability of both pyloric and gastric mill activity [56,57]. Removal of neuromodulation also causes changes severe enough to prevent functional activity when inputs are restored [58].

It is challenging to pry apart the roles that activity-dependent or neuromodulator-dependent mechanisms play in these changes, but two lines of recent studies show that neuromodulators contribute substantially. The first set of studies shows that neuromodulatory input plays an important role in the long-term maintenance of the cell type-specific co-variation of many ionic currents, because this co-variation can be lost or changed after removal of neuromodulators [54,55,59]. These effects depend on cell identity, as they differ across cell types [55]. The second line explores the prolonged effects of tonic nanomolar levels of dopamine (DA) on network activity. In pyloric follower neurons like the lateral pyloric (LP) neuron, the balance between the transient potassium current I_A and the

hyperpolarization-activated current I_h plays an important role in determining the onset phase of activity. DA tone, over hours, maintains the levels of I_A, thereby stabilizing phase, and counteracting reduction of I_A in response to acute micromolar application of DA [60]. Interestingly, acute reduction of I_A and other network influences of μM DA change the bursting activity of the LP neuron in a manner that results in an activity and DA tone-dependent decrease in I_h, thereby homeostatically recovering control levels of LP activity [61]. The second messenger pathway as well as components of the transcriptional and translational regulation underlying these effects have recently been identified [62,63, 64*].

Robustness to temperature changes

Challenges to the need for stable circuit activity can also come from changing physical parameters such as temperature. Lobsters and crabs are poikilothermic and experience substantial temperature changes. Similar network activity is achieved with variable sets of synaptic and intrinsic properties across individuals, and temperature differentially affects biological processes with a wide range of Q_{10} values. Therefore, temperature change could have highly variable consequences. Nevertheless, the pyloric pattern frequency consistently tracks temperature linearly over a wide range, with stable phase relationships between neurons, only revealing individual differences at high temperatures when the rhythm crashes. This is true for the full circuit *in vitro* [65] and *in vivo* [66], as well as the isolated pacemaker kernel [67]. Pacemaker models can produce similar activity with many possible combinations conductance magnitudes and Q_{10} values, robust over a range of temperatures [68]. Neuromodulation may play an important part in conferring robustness to the circuits. Activity levels of modulatory neurons increase with temperature, and the resulting enhancement of peptide-evoked I_{MI} can rescue activity of the gastric mill circuit by counteracting temperature-induced increase in leak currents [69*]. Mass spectrometry-based approaches show that the neuropeptide complement changes with increasing temperature [70].

Conclusions

The STNS continues to be a valuable model for uncovering fundamental principles underlying circuit dynamics. The recent work in this system discussed here highlights the need to consider how mechanisms that span different time scales interact (Fig. 3). The dynamics of intrinsic neuron properties and synapses are constantly shaped by neuromodulation, and are continuously tuned by long-term regulatory mechanisms that maintain stable circuit function. To produce proper circuit output, these regulatory mechanisms do not rely on fixed parameter combinations, but use correlative rules which can result in many different solutions. Yet those solutions must incorporate consistent responses to neuromodulators which, themselves, can be involved in long-term regulatory mechanisms. Such findings are likely generalizable to any circuit and have been greatly aided by, or indeed only been possible because of the numerical simplicity of the STG circuits. However, both the dynamics and the many different possible parameter combinations demonstrate that even such small circuits are anything but simple, which should inform any attempts at functionally dissecting larger circuits.

Acknowledgments

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Highlights

- Analysis of small circuits reveals interacting dynamics at different time scales.
- Synaptic and intrinsic properties, neuromodulation, and long-term regulation interact.
- Neuromodulation ties together short-term flexibility and long-term stability.
- Circuit parameters are variable, but correlated to ensure stable function.

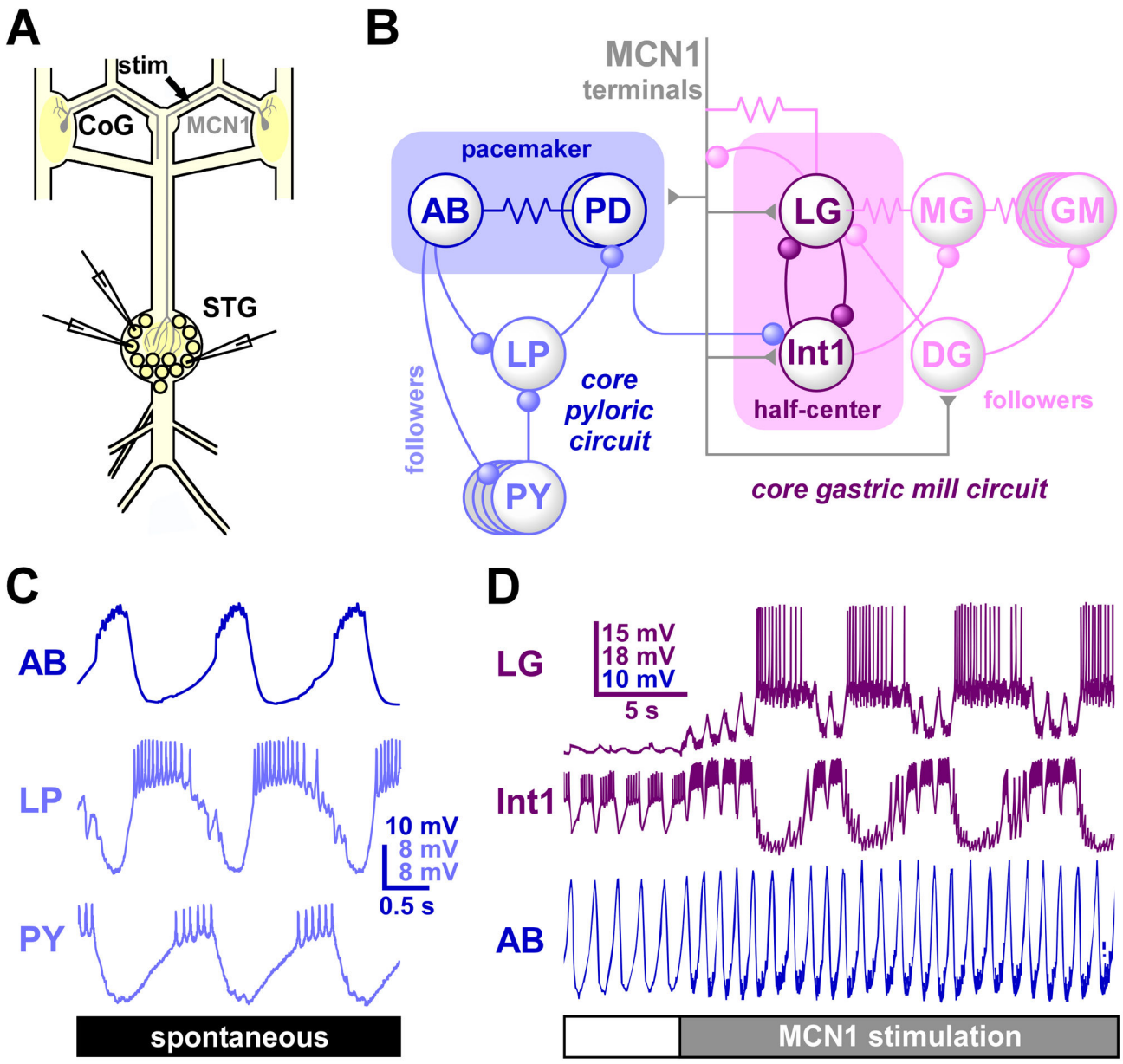

Figure 1.

The pyloric and gastric mill central pattern generating circuits of the stomatogastric ganglion. **A:** Schematic of the isolated STNS. The STG contains the pyloric and gastric mill circuits. The commissural ganglia (CoG) contain the cell bodies of projection neurons like the modulatory commisural neuron 1 (MCN1), which project to the neuropil of the STG. **B:** The core pyloric and gastric mill circuit diagrams. Not all cells types and synapses are shown. Inhibitory chemical synapses are shown as circles, electrical coupling as resistor symbols, and excitatory inputs from MCN1 as triangles. Rhythm generation is based on intrinsic oscillatory properties of the pacemaker kernel in the pyloric circuit, and on reciprocal inhibitory connections between non-oscillatory neurons (half-center) in the gastric mill circuit. Note that both circuits are interconnected by direct synapses and through

feedback to the terminals of projection neurons. **C:** The typical tri-phasic pyloric pattern. In each cycle, a pacemaker burst is followed by neurons burst in two different phases, in rebound from pacemaker inhibition. **D:** The bi-phasic gastric mill rhythm is often not spontaneously active, but can be activated by stimulating modulatory projection neurons like MCN1. Note that the interconnection between both circuits leads to substantial pyloric modulation of the much slower gastric mill neuron bursting. The pyloric pacemaker neuron AB is shown as a reference for pyloric timing. **A**, **B**, & **D** are modified from reference [9]; **C** is modified from reference [8].

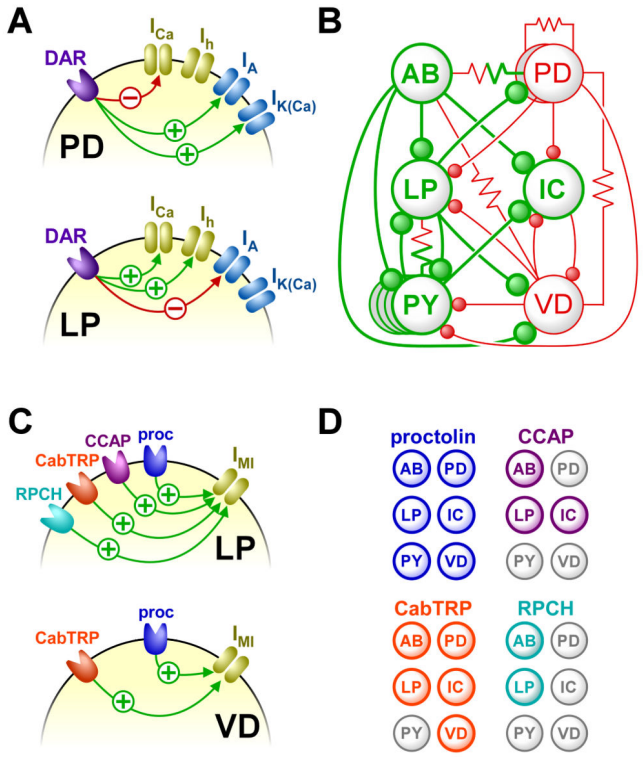

Figure 2.

Different organizing principles underlying circuit modulation by biogenic amines and neuropeptides. **A:** In different cell types, activation of dopamine receptors (DAR) can affect the gating properties of different subsets of ion channels, and the effects can have a different sign. Ion channels giving rise to inward currents are shown in yellow, and those giving rise to outward currents in blue. **B:** The sum effects of the diverse cellular loci of dopamine actions are functional enhancements (green) or recuctions (red) of excitability in all pyloric neurons and strength of all pyloric synapses. **C:** Neuropeptide modulation affects a limited number of intracellular targets. Different neuropeptides all converge on the same voltage-gated inward current (I_{MI}), but different cell types respond to a different subset of neuropeptides. RPCH: red pigment concentrating hormone; CabTRP: *Cancer borealis* tachykinin-related peptide; CCAP: crustacean cardioactive peptide; proc: proctolin. **D:** Despite the convergence of neuropeptide effects on the same subcellular target, the different subsets of circuit neurons affected by each neuropetide give rise to divergent effects on circuit activity. **A** & **B** are modified from reference [16], **C** & **D** are modified from references [27] and [32].

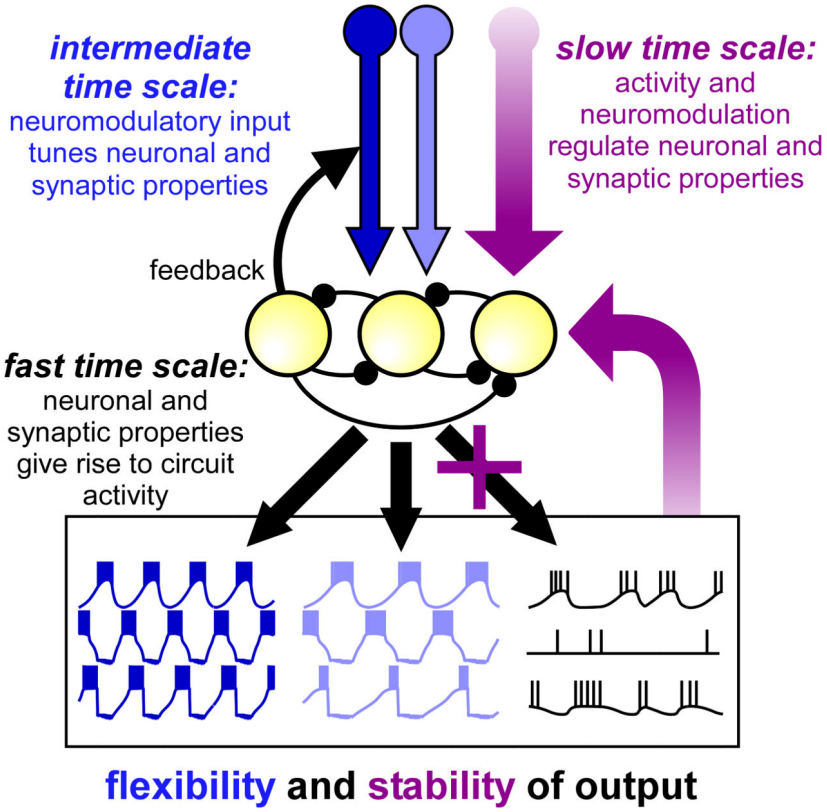

Figure 3.

Schematic of interactions of different regulatory mechanisms that affect circuit operation at different time scales. At the fast time scale, neuronal and synaptic properties give rise to circuit activity. At the intermediate time scale, neuromodulators convey flexibility, as different neuromodulators can tune neuronal and synaptic properties to generate different circuit outputs. Circuit activity itself can shape input patterns from modulatory neurons through feedback connections. At the slow time scale, long-term regulatory mechanisms dependent on neuronal activity and the presence of neuromodulators convey stability of circuit output and prevent circuit crashes.