



# Chronic DREADD Isn't As Bad As It Sounds

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## Long-Term Chemogenetic Suppression of Spontaneous Seizures in a Mouse Model for Temporal Lobe Epilepsy

Desloovere J, Boon P, Larsen LE, et al. *Epilepsia*. 2019;60(11):2314-2324. doi:10.1111/epi.16368.

**Objective:** More than one-third of patients with temporal lobe epilepsy (TLE) continue to have seizures despite treatment with antiepileptic drugs, and many experience severe drug-related side effects, illustrating the need for novel therapies. Selective expression of inhibitory Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) allows cell-type-specific reduction of neuronal excitability. In this study, we evaluated the effect of chemogenetic suppression of excitatory pyramidal and granule cell neurons of the sclerotic hippocampus in the intrahippocampal mouse model (IHKA) for TLE. **Methods:** Intrahippocampal IHKA mice were injected with an adeno-associated viral vector carrying the genes for an inhibitory DREADD hM4Di in the sclerotic hippocampus or control vector. Next, animals were treated systemically with different single doses of clozapine-N-oxide (CNO; 1, 3, and 10 mg/kg) and clozapine (0.03 and 0.1 mg/kg), and the effect on spontaneous hippocampal seizures, hippocampal electroencephalography (EEG) power, fast ripples (FRs), and behavior in the open field test was evaluated. Finally, animals received prolonged treatment with clozapine for 3 days, and the effect on seizures was monitored. **Results:** Treatment with both CNO and clozapine resulted in a robust suppression of hippocampal seizures for at least 15 hours only in DREADD-expressing animals. Moreover, total EEG power and the number of FRs were significantly reduced. Clozapine-N-oxide and/or clozapine had no effects on interictal hippocampal EEG, seizures, or locomotion/anxiety in the open field test in non-DREADD epileptic IHKA mice. Repeated clozapine treatment every 8 hours for 3 days resulted in almost complete seizure suppression in DREADD animals. **Significance:** This study shows the potency of chemogenetics to robustly and sustainably suppress spontaneous epileptic seizures and pave the way for an epilepsy therapy in which a systemically administered exogenous drug selectively modulates specific cell types in a seizure network, leading to a potent seizure suppression devoid of the typical drug-related side effects.

## Commentary

Over the past 15 years, the explosion in tools to selectively modulate activity of defined populations of neurons has raised substantial promise and interest for the treatment of epilepsy. Preclinical tools such as optogenetics, which exploits light-sensitive ion channels, or chemogenetics, which employs designer receptor/drug combinations, have shown efficacy in a wide array of models of epilepsy. For a recent review of these approaches, see Walker and Kullmann.<sup>1</sup>

The use of optogenetics, however, has far outstripped the use of chemogenetics. While optogenetic approaches offer real-time neuromodulation, chemogenetic approaches rely on designer drug delivery, which is inherently slower and thus not amenable to real-time neuromodulation. This has, in part, led to more restricted use of chemogenetics in preclinical studies of epilepsy. However, while the reduced temporal resolution of chemogenetic approaches may be a weakness in some regards, the approach offers several strengths<sup>2</sup>: (1) drug can be

delivered in a minimally invasive manner, whereas optogenetics requires indwelling hardware, (2) larger volumes of tissue and/or distributed network nodes may be easier to target as the volume of tissue modulated isn't limited by light delivery, and (3) chemogenetics avoids tissue heating that can be seen with optogenetics.

One of the earliest reports to describe chemogenetic manipulations used an "excitatory" approach in which DREADDs (Designer Receptors Exclusively Activated by Designer Drugs) were expressed in forebrain excitatory neurons; activation of these receptors by administration of clozapine-n-oxide (CNO), a metabolite of the antipsychotic drug clozapine, produced repeated limbic seizures in transgenic mice.<sup>3</sup> Since the original characterization of these tools, DREADDs have been used to attenuate seizures in an array of models including focal neocortical seizures,<sup>4</sup> amygdala kindled seizures,<sup>5</sup> and seizures following systemic pilocarpine<sup>6</sup> or intrahippocampal kainate.<sup>7</sup> As is often the case with emerging technologies, our



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understanding of the limitations of reagents increases with time—in the intervening years, it has become clear that CNO can cause effects in and of its own right<sup>8</sup> and that its utility as a drug target is likely limited due to poor blood–brain barrier penetrance.<sup>9</sup> As a result, alternative approaches, including the use of clozapine,<sup>9</sup> and more recently olanzapine<sup>10</sup> as high-affinity DREADD agonists have been suggested.

Desloovere and colleagues<sup>11</sup> employed a chemogenetic strategy (inhibiting excitatory neurons in the hippocampus in mice following intrahippocampal kainate injection) and used clozapine as their drug of choice. Clozapine offers some advantages over CNO, including better brain penetrance, and some have suggested that CNO may be metabolized into clozapine, which in turn is actually responsible for CNO effects on DREADDs.<sup>9</sup> As clozapine is an approved antipsychotic agent, the use of low-dose clozapine may be more readily translatable to humans as compared to CNO (however, note that clozapine use may be limited in humans as it can cause severe neutropenia).

In a head-to-head comparison of CNO and clozapine, these authors found equivalent suppression of electrographic seizure activity in mice. They also reported significantly greater suppression of seizure activity with a higher dose (0.1 mg/kg) of clozapine as compared to a lower dose (0.03 mg/kg). These effects were evident for up to 15 hours following administration of a single dose, which suggests minimal receptor desensitization (or alternatively, a large receptor reserve), which is precisely the profile that would be necessary for translational utility.

To evaluate longer term seizure suppression, Desloovere et al<sup>11</sup> carried out monitoring during 36-hour repeated clozapine dosing. As in the prior studies evaluating DREADD-mediated seizure control in spontaneous models,<sup>6,7</sup> Desloovere used an on-off-on design. The authors found a near complete suppression of electrographic seizure activity during the 36-hour “off” period. This profile is complementary to that reported by Wang and colleagues<sup>7</sup> who found that chemogenetic activation of parvalbumin interneurons or chemogenetic inhibition of hippocampal pyramidal neurons robustly suppressed behavioral seizures in the intrahippocampal kainate model in mice. The seizures in the intrahippocampal kainate model are predominantly electrographic, although a subset of electrographic events are convulsive.<sup>12</sup> Unfortunately Desloovere and colleagues did not report effects on behavioral seizure activity, which would have served as a strong complement to their findings.

Interestingly, Desloovere et al<sup>11</sup> as well as Wang et al<sup>7</sup> found a profile suggestive of a posttreatment rebound excitability, with the frequency of seizure events increasing over baseline after termination of chemogenetic therapy. While in neither study did this reach the level of statistical significance, this does raise the possibility that even moderate-term chemogenetic therapy may produce cellular and network compensation, and as with many antiseizure therapies, discontinuation may need to be gradual.

In addition to the effects on seizure activity, Desloovere et al<sup>11</sup> also found that CNO treatment decreased both hippocampal power and fast ripple prevalence in DREADD-positive animals, but not in controls. The authors did not report effects of chemogenetic therapy on normal ripple activity; thus, it is unclear if this approach would spare or impact normal ripple oscillations, which have been associated with memory consolidation processes.<sup>13</sup> Abolishing fast ripples and seizures while sparing normal ripple activity seems an ideal strategy to avoid cognitive impairment. Above and beyond DREADD-mediated effects on normal physiology, clozapine (and CNO) may have *DREADD-independent* actions.<sup>8</sup> Reassuringly, Desloovere et al<sup>11</sup> found no effects of clozapine or CNO delivery on hippocampal activity or seizures in animals that did not express DREADD receptors and similarly found no effect of either drug in the open field task. While by no means a comprehensive assessment of potential off-target effects, these data nonetheless strengthen the case for this approach.

The results of this study provide strong preliminary rationale to continue pursuing chemogenetics as a potential approach for human TLE. The effects of these chemogenetic approaches are similar in magnitude to those reported using optogenetics in the same model.<sup>14</sup> Follow-up proofs-of-concept that characterize additional DREADD strengths and potential side effects will help to determine whether this approach can be employed chronically without the risk of rebound, to continue to evaluate more efficacious designer drugs, and to ensure that such seizure suppression occurs without interfering with normal hippocampus-dependent processes.

The accumulating evidence for chemogenetic approaches in temporal lobe epilepsy models raises a few questions about translation. First, how well will DREADD technology work in the human brain? A growing number of studies have used chemogenetics in nonhuman primates,<sup>15</sup> and while the success rate has certainly been lower than in rodents, these studies suggest that it is feasible to scale the approach to a larger brain.

In sum, this study indicates that chemogenetic suppression of excitatory neurons at the seizure focus is sufficient to reduce seizure burden in a robust and prolonged manner. It adds more translational pharmacology (ie, clozapine) to a growing literature employing chemogenetics to control seizures and, while still far removed from clinical use, underscores the intriguing translational potential of this methodology.

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