The Gs-Linked Receptor GPR3 Maintains Meiotic Arrest in Mammalian Oocytes
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I find this highly unlikely. Biological compensation mechanisms (35, 36) are likely to limit the maximal effect of cocaine on neural systems, including the value representation. This can be modeled in a number of ways, one of which is to include a global effectiveness-of-dopamine factor, which multiplies all R(s) and D(s) terms. If this factor decreased with each drug receipt, the values of all states would remain finite. Simulations based on an effectiveness-of-dopamine factor that decreases exponentially with each drug receipt (factor = 0.99^n, where n is the number of drug receipts) showed similar properties to those reported here, but the values of all states remained finite.

Another important issue in reinforcement learning is what happens when the reward or drug is removed. In normal TDRL, the value of states leading to reward decay back to zero when that reward is not delivered (16). This follows from the existence of a strongly negative δ signal in the absence of expected reward. Although firing of dopamine neurons is inhibited in the absence of expected reward (16), the inhibition is dramatically less than the corresponding excitation (7). In general, the simple decay of value seen in TDRL (6, 39) does not model extinction very well, particularly in terms of reinstatiation after extinction (40). Modeling extinction (even for natural rewards) is likely to require additional components not included in current TDRL models, such as state-space expansion.

A theory of addiction that is compatible with a large literature of extant data and that makes explicitly testable predictions has been deduced from two simple hypotheses: (i) dopamine serves as a reward-error learning signal to produce temporal-difference learning in the normal brain and (ii) cocaine produces a phasic increase in dopamine directly (i.e., neuropharmacologically). A computational model was derived by adding a noncompensable δ signal to a TDRL model. The theory makes predictions about human behavior (developing inelasticity), animal behavior (resistance to blocking), and neurophysiology (dual dopamine signals in experienced users). Addiction is likely to be a complex process arising from transitions between learning algorithms (3, 20, 22). Bringing addiction theory into a computational realm will allow us to make these theories explicit and to directly explore these complex transitions.

References and Notes

14. See materials and methods on Science Online.
34. C. W. Bradberry, Neuroscientist 8, 315 (2002).
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Supporting Online Material

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Materials and Methods
Figs. S1 to S7
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The Gs-Linked Receptor GPR3 Maintains Meiotic Arrest in Mammalian Oocytes

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Mammalian oocytes are held in prophase arrest by an unknown signal from the surrounding somatic cells. Here we show that the orphan Gs-linked receptor GPR3, which is localized in the oocyte, maintains this arrest. Oocytes from Gpr3 knockout mice resume meiosis within antral follicles, independently of an increase in luteinizing hormone, and this phenotype can be reversed by injection of Gpr3 RNA into the oocytes. Thus, the GPR3 receptor is a link in communication between the somatic cells and oocyte of the ovarian follicle and is crucial for the regulation of meiosis.

Meiosis, which reduces the oocyte’s chromosome number in preparation for fertilization, begins long before fertilization occurs. In most species, including mammals, DNA replication, entry into meiosis, and chromosomal recombination occur early in oogenesis, but then at late prophase, meiosis arrests. Much later, shortly before ovulation, meiosis resumes: the nuclear envelope breaks down, the chromosomes condense, and a metaphase spindle is formed. In vertebrates, this occurs in response to luteinizing hormone (LH) from the pituitary, which acts on the somatic (granulosa) cells that surround the oocyte in the ovarian follicle (1, 2).

Throughout much of mammalian oogenesis, prophase arrest is maintained by inherent factors in the oocyte and correlates with low levels of activity by cell cycle regulatory proteins, including cyclin B and CDK1 (1). However, once the oocyte reaches its full size and an antral space begins to form between the granulosa cells, prophase arrest in the oocyte becomes dependent on unidentified signals from the granulosa cells. Oocytes that are removed from antral follicles resume meiosis spontaneously (3, 4).

The maintenance of prophase arrest in oocytes within antral follicles requires the activity of signaling molecules within the...
In particular, the heterotrimeric G protein $G_\alpha$ is required; injection of oocytes with an inhibitory $G_\alpha$ antibody, or a dominant-negative form of $G_\alpha$, causes meiosis to resume within the follicle (5, 6). $G_\alpha$ stimulates adenylyl cyclase in the oocyte to keep cyclic

adenosine monophosphate (cAMP) elevated (7), and this results, through activation of protein kinase A and subsequent incompletely understood steps, in the inhibition of the cyclin B–CDK1 complex that drives the prophase-to-metaphase transition (8–11). Because $G_\alpha$, by itself has no detectable constitutive activity (12), it is likely that a receptor in the oocyte membrane is required to keep $G_\alpha$ active.

To identify such a receptor, we searched an expressed sequence tag (EST) database derived from a cDNA library of fully grown prophase-arrested mouse oocytes (13), looking for proteins predicted to be G protein–coupled receptors (GPCRs) because of their 7-transmembrane structure. Of the ~1000 mammalian genes that encode 7-transmembrane proteins, 15 were found in the oocyte EST database (table S1). One of these proteins, the orphan receptor GPR3, was of particular interest because it elevates cAMP when expressed in a variety of cultured cells (14–16), implying that GPR3 activates $G_\alpha$.

GPR3 RNA is found predominantly in the ovary, testis, and brain; it has not been found elsewhere, except at very low levels in kidney and lung (14, 17–19). In situ hybridization of mouse ovarian sections showed that GPR3 RNA is localized in the oocyte (Fig. 1A), and reverse transcription polymerase chain reaction (RT-PCR) measurements indicated that GPR3 RNA expression is ~14 times higher in the oocyte than in the surrounding somatic cells (Fig. 1B).

To investigate whether the GPR3 receptor is required to maintain meiotic prophase arrest in mouse oocytes, we examined histological sections of ovaries from Gpr3 knockout mice (13). The Gpr3$^{–/–}$ animals were indistinguishable from Gpr3$^{+/+}$ animals in external morphology, growth, and activity. Their ovaries were of normal size and had a distribution of preantral, early antral, and antral follicles similar to that seen in Gpr3$^{+/+}$ mice (fig. S1). The appearance and organization of the somatic cells in the Gpr3$^{–/–}$ follicles was similar to that in the Gpr3$^{+/+}$ follicles (Figs. 2, A and B; 3, A and B; and fig. S1).

However, although all of the oocytes within antral follicles of Gpr3$^{+/+}$ ovaries were in prophase, with an intact nuclear envelope and nucleolus (Fig. 2A; Fig. 3, A and D; and fig. S2), most of the oocytes within antral follicles

![Image of ovarian follicles](https://example.com/ovarian_follicles.png)

Fig. 1. Gpr3 RNA is localized in the oocyte versus the somatic cells of antral follicles. (A) In situ hybridization of an ovarian section from a 22-day-old eCG-primed mouse, using a 33P-labeled Gpr3 antisense riboprobe. Left: A transmitted light image of a follicle. Right: A reflected light image of the silver grains in the overlying autoradiographic emulsion. Scale bar, 100 μm. (B) RT-PCR comparing the relative expression of Gpr3 RNA in oocytes and somatic cells dissected from antral follicles. RT-PCR for Gpr3 was performed with cDNA derived from the indicated amounts of total RNA from each tissue. Normalized to total RNA, oocytes contained 14 ± 7 times as much Gpr3 RNA as somatic cells (mean ± SD for six RT-PCR experiments with cDNA from two separately collected sets of oocytes and somatic cells) (13).

![Image of follicles](https://example.com/follicles.png)

Fig. 2. Spontaneous resumption of meiosis in an oocyte within a Gpr3$^{–/–}$ antral follicle (adult mouse). (A) An antral follicle from a control Gpr3$^{+/+}$ ovary. The oocyte is arrested in prophase. (B) An antral follicle from a Gpr3$^{–/–}$ ovary. The oocyte has metaphase II chromosomes and a polar body. The right panel is an enlarged view of the left. Mitosis is ongoing in the somatic cells. Scale bars, 100 μm.

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of Gpr3−/− ovaries contained metaphase chromosomes, indicating that meiosis had resumed (Fig. 2B; Fig. 3, B and D; and fig. S2). For these studies, we primarily used ovaries from prepubertal (22- to 24-day-old) mice to avoid the complexity of the cycling adult ovary (18). In the prepubertal ovaries, 82% of the antral follicles contained oocytes that had resumed meiosis, compared with 0% in Gpr3+/+ controls (Fig. 3D). The one adult Gpr3−/− ovary that we analyzed also showed that the majority of oocytes in antral follicles had resumed meiosis (Fig. 2B and fig. S2).

Among the Gpr3−/− antral follicles containing oocytes that had resumed meiosis, 42% were clearly at metaphase II, as indicated by the presence of a polar body (Figs. 2B and 3B). Oocytes that contained a metaphase spindle but lacked a visible polar body could have been at metaphase I or at metaphase spindle but lacked a visible polar body (Figs. 2B and 3B). Oocytes that contained a metaphase spindle, although they did not resume meiosis were competent to contain oocytes that had resumed meiosis, at the time of isolation or 5 hours later (data from early antral follicles in adult mice). Possibly another Gs-linked receptor functions to maintain arrest in the early antral stage, oocytes become dependent on the somatic cells (4) and on cAMP (7, 20), for the maintenance of prophase arrest. In the early antral follicles of prepertual Gpr3−/− mice, 37% of the oocytes had resumed meiosis, as indicated by the presence of condensed chromosomes (Fig. 3, C and D); within the early antral category, oocytes in larger follicles showed a larger fraction of meiotic resumption (table S3). Most of these oocytes showed a metaphase spindle, although a few appeared to be at prometaphase; polar bodies were not observed (fig. S2 gives results from early antral follicles in adult mice).

To determine whether the oocytes in Gpr3−/− early antral and antral follicles that did not resume meiosis were competent to do so if removed from the follicle, we dissected follicles of comparable size ranges from Gpr3−/− ovaries (13). Thirty-six percent of the oocytes from Gpr3−/− early antral follicles had resumed meiosis at the time of isolation, and only a few additional oocytes had resumed meiosis by 5 hours after isolation (Fig. 3E). Thus, during follicle development, the maintenance of meiotic arrest becomes dependent on the GPR3 receptor at close to the same time that arrest becomes dependent on the somatic cells. Of the oocytes from Gpr3−/− antral follicles, 89% had resumed meiosis at the time of isolation, and this percentage increased to 99% by 5 hours after isolation (Fig. 3E). Possibly another Gs-linked receptor functions to maintain arrest in the small subgroup of Gpr3−/− oocytes that do not resume meiosis in their antral follicles.

The resumption of meiosis in Gpr3−/− follicles did not appear to result from an increase in LH, as could occur in a cycling adult mouse or potentially even in a prepertual mouse if pituitary function was affected by the absence of GPR3. LH action on the mural granulosa cells causes extracellular matrix material to be deposited around the cumulus granulosa cells, resulting in an expansion of the cumulus mass in preparation for ovulation; cumulus expansion is evident by the time the oocyte reaches metaphase I (21, 22) (Fig. 4A). However, no spontaneous cumulus expansion was seen in the Gpr3−/− follicles (Figs. 2B and 3B). Likewise, there was no spontaneous expansion of the follicle diameter (table S4), as is seen in response to LH (23). Thus, the presence of chromosomes aligned on metaphase I or II spindles in an unexpanded follicle with an unexpanded cumulus mass is not consistent with a response of the follicle to LH. Expression of an LH-dependent gene that encodes one of the proteins produced during cumulus expansion, chondroitin sulfate proteoglycan.
Fig. 4. Resumption of meiosis in Gpr3\(^{-/-}\) ovaries is not due to elevation of LH and is prevented by injection of Gpr3 RNA into the oocyte. (A) For comparison with the Gpr3\(^{-/-}\) follicle shown in Fig. 3B, cumulus expansion in an antral follicle from a 22-day-old wild-type mouse injected 9 hours previously with the LH receptor agonist, hCG. Metaphase I oocytes reached metaphase II without cumulus expansion. (B) There was no increase in expression of an LH-dependent gene, Csp2, when Gpr3\(^{-/-}\) and Gpr3\(^{+/+}\) ovaries were compared. RT-PCR for Csp2 was performed using cDNA derived from the indicated amounts of ovary RNA. Similar results were obtained in two experiments. For comparison, the lower panels show the increased expression of Csp2 RNA in a Gpr3\(^{+/+}\) ovary collected 4 hours after injection of the mouse with hCG, versus an unstimulated Gpr3\(^{+/+}\) ovary. LH receptor stimulation increased expression of Csp2 RNA by a factor of \(-25\). Previous work has shown that Csp2 RNA increases by 2 hours after LH receptor stimulation and remains elevated for 16 hours (22). (C) Rescue of the ability to maintain meiotic arrest by injection of Gpr3 RNA into prophase-arrested Gpr3\(^{-/-}\) oocytes within preantral or very early antral follicles. After 4 days in culture, when antrum formation was occurring in most follicles, oocytes were isolated to determine if meiosis had resumed. Numbers above the bars indicate how many follicles and mice were analyzed. The percent meiotic resumption in Csp2 RNA-injected Gpr3\(^{+/+}\) oocytes was significantly different from that in un.injected oocytes and in control oocytes injected with RNA encoding red fluorescent protein (\(P = 0.0009\) and 0.007, respectively, Fisher’s exact test).

2 (CSP2 or versican V0) (22), was also not elevated in Gpr3\(^{-/-}\) ovaries (Fig. 4B).

The resumption of meiosis in Gpr3\(^{-/-}\) ovaries cannot be attributed to follicle atresia, because all of the Gpr3\(^{-/-}\) follicles included in Fig. 3D appeared to be morphologically healthy, and mitosis was ongoing in the granulosa cells (Figs. 2B and 3B). A normal number of ovaeggs was found in the oviducts of a Gpr3\(^{-/-}\) mouse that had been injected 13 hours previously with the LH receptor agonist, human chorionic gonadotropin (hCG) (24), and sections of an ovary from an adult Gpr3\(^{-/-}\) mouse showed corpora lutea (fig. S1). Thus, the pathways leading to ovulation and luteinization appear to be unimpaired in Gpr3\(^{-/-}\) mice. The only detected defect in the Gpr3\(^{-/-}\) ovary was the absence of the ability to maintain meiotic prophase arrest.

The predominant expression of Gpr3 RNA in the oocyte versus the somatic cells of the follicle, and the dependence of meiotic arrest on oocyte G\(_s\) (5, 6), support the conclusion that it is the GPR3 receptor in the oocyte, rather than in the somatic cells, that is primarily required to maintain meiotic arrest. To examine whether the spontaneous resumption of meiosis could be prevented by introducing GPR3 protein into Gpr3\(^{-/-}\) oocytes, we injected prophase-arrested Gpr3\(^{-/-}\) oocytes within preantral or very early antral follicles with Gpr3 RNA and then cultured them under conditions that allowed antral formation (25) (Fig. 4C and fig. S3). After the culture period, about half of control Gpr3\(^{-/-}\) oocytes had resumed meiosis, but only 11% of the Gpr3\(^{-/-}\) oocytes that had been injected with Gpr3 RNA had resumed meiosis (Fig. 4C). The reversal of the knockout phenotype by Gpr3 RNA injection into the oocyte further indicates that the GPR3 receptor that maintains meiotic arrest is located primarily in the oocyte itself.

Although GPR3 has some ligand-independent activity (14–16), additional activation of GPR3 in the oocyte by an agonist from or on the granulosa cells could explain why meiosis remains arrested only if the oocyte is surrounded by granulosa cells. A GPR3 ligand that increases G\(_s\) activation, rather than in the somatic cells, that is required to maintain meiotic arrest provides clues about how LH might relieve the arrest and reinitiate meiosis.

References and Notes

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