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Conditioned taste aversion as a learning and memory paradigm

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Abstract

Conditioned taste aversion (CTA) is a well established learning and memory paradigm in rats and mice that is considered to be a special form of classical conditioning. Rodents — as well as many other species including man — learn to associate a novel taste (CS) with nausea (US), and as a consequence avoid drinking fluid with this specific taste. In contrast to other types of classical conditioning, even CS–US intervals lasting several hours lead to an aversion to the gustatory CS. With increasing CS–US delay duration, however, the aversion against the CS gradually decreases. Mice differ from rats in their reaction to the CS as well as the US. They tolerate a much higher concentration of saccharin and they do not show any clear signs of nausea when injected with the US. Advantages of this task are its relative independence of motor behavior, well described pathways for the CS and partly the US, and the wealth of available anatomical and pharmacological data implying several brain structures (e.g. parabrachial nucleus, amygdala, insular cortex), neurotransmitters and their receptors (e.g. cholinergic system, NMDA-receptors), and cellular processes (e.g. expression of immediate early genes, *Ras–MAP* kinase signaling pathway, CREB phosphorilation, protein tyrosine phosphorilation, protein synthesis) in CTA. The CTA paradigm has also been successfully used to phenotype mouse mutants.

Keywords: Transgenic mice; Knockout; Method; Behavioral phenotyping

1. Introduction

To survive in a world with varying supplies of different foods animals have to learn which are safe and which are not safe to eat. Most foods are characterized by a specific flavor, i.e. a unique combination of taste and smell. Thus, learning which flavors signal 'safe to eat' and which 'causes nausea' is important for making use of all the safe foods while at the same time avoiding potentially hazardous ones. Taste is an especially critical information because if it signals 'causes nausea' the animal has a last chance to refrain from eating the food.

A large variety of mammalian species can develop aversions against food or liquids of specific tastes in the wild as well as in the laboratory (introduced by Garcia in 1955 [37]). In the laboratory, these tastes can be initially neutral; but if ingestion of such a flavored substance is followed by a nausea-inducing treatment it will be avoided in the future. However, if the ingestion is followed by an injection of toxic substances not directly affecting the gastro-intestinal tract such as strychnine or cyanide [49,84], or if it is followed by electric shock [38], no aversion develops.

Acquisition of taste aversion represents a form — and follows the rules — of classical conditioning with the sickness-inducing substance being the unconditioned stimulus (US) and the nausea the unconditioned reaction (UR). The taste stimulus becomes the conditioned stimulus (CS) eliciting a not directly observable conditioned reaction (CR); avoiding the CS is usually taken as a sign of a successfully established conditioned reaction, and the animal is said to have developed a conditioned taste aversion (CTA). Besides avoidance of the CS (e.g. sucrose solution), mimetic signs of aversion after forced consumption of the CS are detectable after conditioning with some USs (e.g. LiCl) but not other USs [94,141]. Mimetic signs elicited by aversive tastes injected into the mouth include lingual, masticatory, and facial musculature [16,40].

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Acquisition of a CTA has been successfully used as a learning and memory paradigm in rats and mice. It is a biologically meaningful paradigm, i.e. it also can be readily observed outside the laboratory under natural conditions. For better control of the experimental situation, fluids instead of solid foods are used and in most cases specific tastes as the CS are preferred over flavors as the CS. Further, two-bottle tests are more sensitive than one-bottle tests to obtain an aversion index [26,41]. CTA has often been used in drug discrimination studies and other studies with an interest in the possible nauseating effect of drugs. We will not deal with this aspect in the present short review. Rather, the focus of the review is on demonstrating the usefulness of CTA as a learning and memory paradigm in mice, specifically to phenotype mouse mutants. Neuroanatomical data are presented and pharmacological/cellular processes involved in CTA are described to allow a better interpretation of results from CTA studies with mutant mice. Older reviews and books dealing with CTA from a different perspective are also available [4,17,20].

2. Behavioral characterization of CTA

Compared to the consumption of familiar tasting food, the consumption of food with a novel taste increases the expression of immediate early genes ([80]) and of proteins involved in the *Ras–MAP* kinase signaling pathway [7,8]. A memory trace for the novel taste will be formed and, depending on the consequences that follow the consumption during the next few hours, this new taste will be associated with the label 'familiar and safe' or — after gastrointestinal malaise — the label 'familiar and not safe'. Thus, the animal likely always forms a memory trace of the new taste, but depending on the consequences labels it differently as either 'safe to eat' or 'causes nausea' (for discussion, see [19]). The opposite of CTA, conditioned taste preference, is also possible when the consequences of consumption are positive for the animal, i.e. when a hungry animal eats food with a high nutritional value (for review, see [115,128]).

When the consumption of an already familiar taste is followed by nausea for the first time, the resulting conditioned taste aversion is attenuated [1,29,101]. Such a CS preexposure effect (also called 'latent inhibition') has been reported for a variety of other classical conditioning paradigms including fear conditioning [44] and eyeblink conditioning [116]. Blocking, another phenomenon of classical conditioning, has also been demonstrated with CTA [34,35]. After having acquired a CTA to taste A, animals did not acquire an aversion to taste B when LiCl is injected after the presentation of taste A and B together. Blocking of CTA has also been observed in mice (unpublished data from our laboratory).

Similar to cued fear conditioning and eye blink conditioning, the pathways for the CS in CTA are well described (see below). The brain sites involved in the mediation of the US depend on the US used. In case of the most common US, LiCl, the major areas activated by the US are well known, although the individual projections are less well described (see below). Similar to fear conditioning, but in contrast to eye blink conditioning, CTA can be acquired in a single trial. This offers a number of advantages, e.g. if one wants to investigate the cellular and/or molecular consequences of learning or manipulate the consolidation of the memory trace. CTA has also the unique advantage of a long CS–US interval that allows to target experimental manipulations to the presentation of the CS or the US only. Finally, measuring the avoidance of a saccharin solution as CS in a choice test between the CS and water is an easily to measure indicator for the strength of the CR.

3. The CS

The gustatory pathways of rodents are well described (for review, see [62,90]). Briefly, gustatory information from the tongue and oral cavity reaches the gustatory zone of the nucleus of the solitary tract (NST) via branches of cranial nerves VII, IX and X. From the NST the information is conveyed to the gustatory area in the parabrachial nucleus of the pons (PbN). Projections from the PbN ascend ipsilaterally to the parvicellular thalamic ventral posteromedial nucleus (VPMpc). A smaller pathway transmits the information to the central amygdala, the bed nucleus of the stria terminalis, and, sparsely, to the lateral hypothalamus and substantia innominata. From the VPMpc gustatory information finally reaches the gustatory neocortex in the agranular insular cortex (ICag). A relatively weak projection also runs from the VPMpc to the amygdala. An important feature of this ascending gustatory system is that it runs in parallel with visceral information. Descending pathways from the ICag reach almost all gustatory relay stations including the VPMpc, central amygdala, PbN, and NTS. Besides the descending afferents from the ICag, the PbN is modulated by the amygdala and the lateral hypothalamus.

Strain as well as sex can affect an animals reaction to the CS. Sex differences in taste preference for glucose and saccharin solutions — the most common CS used in CTA studies—have been reported [22,132], and this preference is sensitive to prenatal experience [69]. Different strains of mice show a substantial variation in their preference for and ability to discriminate between different tastes including sweet solutions (see, [5,32,58,88,89]).

4. The US

A number of different drugs were successfully used as the US, some of them probably by acting directly on the brain such as amphetamine, apomorphine, or nicotine [6,52,76,127]. However, the most common drug used as the US when memory mechanism of CTA are investigated is lithium chloride (LiCl). Systemically injected (or consumed in the drinking water) LiCl induces nausea by activating vagal and splanchnic afferent nerves [87]. The central neuroanatomical projections mediating nausea are still poorly described. However, several recent studies using expression of immediate early genes as a marker for increased brain activity revealed sites activated by a systemic injection of LiCl [63,122,139] or other drugs that can serve as US [109]. A change in *c-Fos*, *Fos*B, *Jun*B, *Zif*/268, and CREM expression was detected in the NTS, PbN, and the central nucleus of the amygdala after administration of LiCl [63,122], and a change in *Fos*, *Zif*/268, and CREM expression in the hypothalamic PVN [63]. An earlier electrophysiological study found increased activity in the basolateral amygdala after systemic injection of LiCl [97]. Thus, as mentioned before, processing of visceral aversive stimuli parallels processing of gustatory information on several levels including the NTS, PbN, VPMpc, amygdala, and insular cortex. At each of these levels an association between CS and US is possible.

Further, the area postrema plays a key role in mediating the nausea-inducing effects of several but not all drugs. It sends, amongst others, pathways to the lateral part of the PbN and the NST, and it receives vagal and glossopharyngeal fibers as well as afferents from hypothalamic regions and the lateral part of the PbN (for review, see [91]). When using LiCl as the US, an area postrema lesion abolished the acquisition of a CTA [10,104], but such a lesion had no effect on the recall of a previously acquired taste aversion [96]. Area postrema lesions also blocked the development of methylscopolamine- and scopolamine-induced but not amphetamine-induced CTA [6,93].

5. Anatomy of CTA

Acquisition of a CTA changes the pattern of activity in response to the gustatory CS in the NTS, PbN, and amygdala. These changes have been detected using electrophysiological [21,138] as well as molecular techniques (i.e. labeling expression of the immediate early gene *c-fos*; [47,48,111,112,125,127,120,136]). Thus, either the input to or the internal organization in these structures must have changed as a consequence of learning.

The importance of the above mentioned and other structure has also been supported by lesion studies. Permanently or transiently inactivating the *PbN* (medial, lateral) impaired the acquisition of a CTA ([3,12,39,50,51,118,119,137]; for review, [98]). Damaging the *amygdala* produced inconsistent results on CTA. Whereas many studies reported impairments in CTA [36,55,68,81,83,110,117,137] others found no such effects [9,33,45]. It has been argued on the basis of excitotoxic lesion experiments that damaging fibers going through the amygdala rather than the amygdala itself is responsible for the observed attenuation of CTA [28]. One possible procedural detail that might affect the outcome of such studies is the conditioning method [114]. Electrolytic as well as excitotoxic lesions of the amygdala impaired CTA when the CS was intraorally infused. However, presenting the CS in bottles led to the development of a CTA that was not impaired by the same type of amygdala lesions.

Lesions of the medial *thalamus* including the VPMpc can attenuate the acquisition of CTA ([66,67,72,137]; but see also [31,100]), and damaging the *ICag* slows taste aversion learning. In their review paper on the involvement of the gustatory neocortex, Braun and colleagues [15] conclude that the ICag is necessary when associative and discriminatory demands of CTA are high. Animals lacking the ICag can acquire a CTA when CSs are intense, the temporal contiguity of CS and US presentations is large and more CS–US pairings are given. Using a reversible lesion technique it could be shown that the initial processing of the taste cue but not the association of the CS with the US depend on an intact telencephalic cortex (for review, see [18]).

Investigations of a possible involvement of the *hippocampus* in CTA have produced conflicting results. In summary, hippocampal and/or fornix lesions affected acquisition of CTA only mildly or not at all [11,82,137]. Observed lesion-induced changes include a slowed extinction of the task [56]. Investigations of a participation of the hippocampus in latent inhibition of CTA have been similarly inconsistent. Lesions of the hippocampus have been reported to reduce, enhance, or have no effect on latent inhibition of CTA [34,77,86,95,99]. Further, animals with dorsal hippocampal lesions do not show the blocking effect, i.e. after having acquired a CTA to taste A animals do not acquire an aversion to taste B when LiCl is injected after the presentation of taste A and B together [34,35].

A selected list of other brain structures that have been implicated in the formation of a CTA includes the hypothalamus [108], the basal forebrain [71,79,55], probably part of the noradrenergic system [14,27,74,53], and — an effect on extinction only — the globus pallidus [24].

6. Pharmacology/cellular processes involved in CTA

Investigations of the role of neurotransmitter systems in CTA provide strong support for a critical role of glutamatergic and cholinergic systems in that kind of classical conditioning. Blockade of *NMDA-receptors* attenuated conditioning of a taste aversion [2,134]. Intraventricular injection of a competitive NMDA-receptor antagonist failed to affect CTA learning [135]. However, it is not clear how much of the injected drug invaded the ICag which is critically involved in acquisition of this task. Intracranial injection of NMDA-receptor antagonists supported the critical role of these receptors in CTA and further suggested that NMDA-receptors in the ICag are critical for CTA acquisition [43,106]. In addition, acquisition of CTA is accompanied by an increased phosphorilation of the NMDA-receptor subunit 2B [106]. Studies with mGluR7 $-/-$ mice indicated that also metabotropic glutamate receptors are involved in the acquisition of a CTA [75].

First evidence for a possible involvement of the *cholinergic system* in CTA came from an atropine study in rats [25]. Injecting — a rather large — dose of atropine before conditioning interfered with the acquisition of CTA: an earlier investigation did not reveal any effects of systemic scopolamine on CTA acquisition [59]. Injecting muscarinic antagonists directly into the Icag — and thereby reducing possible US effects of the drug — attenuated the development of CTA [85]. Further, the above mentioned phosphorylation of the NMDA-receptor subunit 2B in the ICag could be induced by locally injecting a cholinergic agonist [105]. Transiently inactivating the nucleus basalis magnocellularis whose projections supply acetylcholine to the neocortex reduced acetylcholine release in the ICag and attenuated the formation of CTA [79]. Cholinergic projections to the amygdala might also be important for successful CTA acquisition [42].

Investigation into the role of other neurotransmitters and hormones yielded varying results of no or small alterations in CTA. Testing mutant mice might contribute new insights into this topic. There is some evidence of a mild impairment of CTA in mice carrying a single mutated allele of the tyrosine hydroxylase gene, a mutation that reduces tyrosine hydroxylase activity to approximately 40% [57]. These data are in agreement with studies reporting slight deficits in CTA acquisition after damage to noradrenergic systems [14,27,74].

Cellular/molecular investigations revealed several intracellular signaling cascades that might be involved to a larger or smaller extent in CTA. Blocking the expression of *c-fos* with injection of antisense oligonucleotides into the central amygdala [64] or brain stem [126] attenuated CTA as did the injection of antisense oligonucleotides against *CREB* [65]. CREB-antisense oligonucleotides injected before retrieval into the amygdala did not reduce the already acquired aversion. Recently, evidence accumulated for a role of the *Ras–MAP kinase* signaling pathway in different forms of neural plasticity (for review, [92]). This pathway also seems to be involved in the development of a CTA. An NMDA and muscarinic receptor mediated activation of some of the proteins participating in this cascade was observed after exposure to a novel taste whereas familiar tastes were ineffective in doing so [7]. Injecting an inhibitor of MAPK/ERK into the ICag attenuated the formation of CTA [8]. Recent studies with intraventricular injection of an inhibitor of MEK in mice potently blocked formation of a CTA [124]. Conversely, when mice acquired a taste aversion phosphorilation of MAP kinase increased in the cortex and amygdala [123].

Injecting *PKC* inhibitors during the CS–US interval into the amygdala or ICag inhibited the formation of a CTA [140]. Injecting PKC inhibitors into gustatory areas of the thalamus had no effect. Similarly, delaying injections into the amygdala or ICag by 4 h were also ineffective. Injecting the PKC inhibitor polymyxin B into the PbN also blocked acquisition of a CTA [13]. However, unspecific effects of the drug might have contributed to this effect. Further evidence for an involvement of PKC in CTA comes from two other studies. Systemic injection of LiCl marginally increases PKC activity in the PbN [60]. Acquisition of CTA activated PKC in the PbN [61]. This activation was characterized by a slow rise peaking at 28 h and a slow decline over the next several days. Transgenic mice deficient in *fyn*, a non-receptor tyrosine kinase, were also impaired in CTA learning [113] as were transgenic mice overexpressing the *urokinase-type plasminogen activator* in the brain [78]. The latter mutation let to an impairment in several learning tasks without significantly affecting sensory and motor capabilities. *Synaptotagmin IV* mutant mice developed a normal CTA [30]. This might be due to the observation that this mutation primarily affected the hippocampus, a structure that seems to have only a minor involvement in CTA learning (see above).

Protein synthesis is a necessary step in the formation of long-term memory [23,121]. Thus, injection of the protein synthesis inhibitor anisomycin into the ICag or amygdala before exposure to the CS attenuated the development of an aversion as measured several days later [107]. Anisomycin administered shortly before the retrieval test did not impair CTA. In accordance with the involvement of protein synthesis in long-term memory formation short-term memory in CTA was protein-synthesis independent [46]. Already earlier studies using intraventricular injections of cycloheximide suggested that the development of a long-term memory for a CTA requires the de-novo synthesis of proteins [129–131].

Table 1
CTA in mutant mice

Mutation	CS [a]	US [a]	CTA [a]	Reference
fyn (−/−)	Almond	LiCl	Normal	[113]
DARPP-32 (−/−)	NaCl	Ethanol	Normal	[103]
Dopamine beta-hydroxylase (−/−)	Saccharine	Ethanol	Delayed extinction	[133]
mGluR7 (−/−)	Saccharine	LiCl	Impaired	[75]
5-HT1B (−/−)	NaCl	Ethanol	Normal	[102]
αMUPA	Saccharine	LiCl	Impaired	[78]
Synaptotagmin IV −/−	Saccharine	LiCl	Normal	[30]
Tyrosine hydroxylase −/+	Sucrose	LiCl	Impaired	[57]
Zif 268 (−/−)	Sucrose	LiCl	Impaired	[54]

[a] CS, conditioned stimulus; US, unconditioned stimulus; CTA, conditioned taste aversion.

7. CTA in mutant mice

As has been shown in rats, mice develop a taste aversion even with a very long CS–US interval of 4 h (unpublished results). With increasing CS–US delay duration, the aversion against the CS gradually decreases also in mice. When the CS was a sweet solution, control mice injected with vehicle that did not induce nausea showed a preference for the CS. This preference, however, varies with the strain used [5,32,58,88,89]. In contrast to rats, mice prefer also higher concentrations of saccharin (0.5%). Again in contrast to rats, mice do not show any clear signs of nausea when injected with the US. The typical sign of nausea in rats, lying-on-belly, is not readily observable causing some difficulties in judging whether or not LiCl induced nausea. Already a few studies with mouse mutants supported the suitability of CTA as a measure to phenotype mouse mutants for possible cognitive deficits. Due to the strain-dependent variability in the preference for saccharin care has to be taken to control for the genetic background of mutants and wild types, a demand that has to be met in all mutant mouse studies [70,73].

Mice have been successfully used to investigate especially cellular processes in taste aversion learning (see section above). The advantage of mice over rats is the increasing availability of mice with targeted changes in specific genes. Recently, such mutant mice have been successfully used to investigate cellular mechanisms involved in the formation of a CTA (Table 1). Ongoing studies in our laboratory also provided promising results with mice carrying a mutation in genes known to be involved in synaptic plasticity on the basis of previous studies.

8. Conclusion

Recently, CTA has been successfully used as a learning and memory paradigm in mice. CTA as a model for classical conditioning has several advantages. It is rapidly established and forms a long-lasting memory trace. In contrast to other types of classical conditioning, even CS–US intervals lasting several hours lead to an aversion to the gustatory CS. The CS can be easily manipulated, and the CR as reflected in an aversion index can be accurately measured. CTA is also relatively independent of motor behavior. Pathways for the CS and to a smaller extent also the US are well described. Finally, a wealth of anatomical and pharmacological data are available implying several brain structures (e.g. amygdala, insular cortex), neurotransmitters and their receptors (e.g. cholinergic system, NMDA-receptors), and cellular processes (e.g. immediate early genes, MAP kinase cascades, protein tyrosine phosphorilation) in the acquisition of CTA.

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